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CONNECTIVE TISSUE COMPONENTS OF THE AVIAN AORTA IN ATHEROSCLEROSIS AND AGING

by

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INTRODUCTION

Atherosclerosis is the most common cause of cardiovascular diseases, and diseases of this category are responsible for more than one-half of all deaths in the U.S. and Western countries. Atherosclerosis is an extremely complex disease involving many processes and is further complicated by the inevitable process of aging of the organism.

The primary emphasis of researchers in the past has been studies involving the lipids and lipid metabolism aspects of atherosclerosis. This is understandable from the observation that the feeding of cholesterol readily induces an experimental lesion in several species and also from the vast amount of epidemiological data collected over the years implicating dietary lipids.

Currently, researchers are looking more to the local degenerative changes of the vascular wall incurred in atherosclerosis. Since this disease manifestation is not restricted to, but certainly more common in older individuals, its development appears to be age-related. This necessitates corresponding knowledge of the vascular changes incurred in the aging process. In this manner perhaps this pathological development can be differentiated from the "normal" aging process.

The purpose of this study was to investigate what changes occur in several connective tissue components of the avian

aorta during aging and spontaneous and induced atherosclerosis. Observations were made on the collagen and elastin content of aortas of male chickens of various ages which had been fed several diets to produce varying degrees of atherosclerotic involvement. Also data were collected on the growth rate, feed consumption, total serum cholesterol, systolic blood pressure and total liver lipids of those respective birds.

REVIEW OF LITERATURE

Atherosclerosis is not a new disease to mankind since it has been described as being present in arteries of Egyptian mummies. It has however, become a disease of major importance in the last 20 years and has demanded the attention of a vast number of researchers. Through the years three major hypotheses have been put forth describing the development of arterial plaques, Constantinides (1965) and Balo (1963). The infiltration theory puts major emphasis on leakage of blood lipids into the arterial wall and their failure to pass on through, whereby they are trapped in the wall. Other workers attribute plaque formation to disturbances in the clotting mechanism in which thrombi form and become integrated into the arterial wall. Finally an alteration in the ground substance of the intima and inflammation of the vascular wall has been postulated as the initial reaction which is followed by lipid deposition or accumulation.

An immense amount of work has been published in recent years on all phases and facets of this arterial disease and this will not be an attempt to present a complete literature review. Several review articles have been published which enable one to be partially familiar with the number of areas.

A book edited by Sandler and Bourne (1963) contains fine discussions of several topics including the role of diet in atherosclerosis, epidemiology of the disease in humans, the

filtration concept of atherosclerosis, vascular dynamics, and natural and experimental atherosclerosis in animals. A book by Mitchell and Schwartz (1965) concerns a necropsy study with emphasis on the thrombus-initiating theory. Connective tissue changes in atherosclerosis is the subject of a review by Balo (1963). Hormonal relationships were covered in conference proceedings edited by Pincus (1959), in a review by Kurland and Freedberg (1960), and by Stamler (1963). Hess (1964) discusses the evaluation of drugs in the treatment of atherosclerosis. The chicken has been widely used as an experimental animal in atherosclerosis research and background support for its applicability is presented in an extensive review by Katz and Stamler (1953) and later by Katz and Pick (1961).

Rose (1967) has presented a review of the literature on the effects of general dietary restriction, protein or energy restriction as it affects serum cholesterol, blood pressure and atherosclerosis. In several experiments conducted with growing cockerels to study the effects of protein and energy restriction it was found that serum cholesterol, serum lipids and liver lipids were increased by protein restriction and decreased by energy restriction when the diets contained added cholesterol. Without added dietary cholesterol only slight increases in serum and liver lipids were noted in the protein restricted birds and neither protein or energy restriction significantly affected serum cholesterol. Blood pressure apparently was more associated with body weight, except for some slight reduction

in the case of birds fed the energy-restricted diets containing cholesterol. Spontaneous aortic atherosclerosis was not significantly influenced by either protein or energy restriction, however, cholesterol-induced atherosclerosis of the thoracic aorta was decreased by energy restriction and increased by protein restriction.

Speers (1965) has reviewed the literature in regard to blood pressure, blood lipids and atherosclerosis in the laying hen. Findings by this author indicated no significant effects were attributable to the levels of protein and energy used in regard to spontaneous atherosclerosis, serum lipids or blood pressure. Trends indicating strain differences in serum lipids and systolic blood pressure were observed.

Nichols (1963) summarized the literature concerning dietary cholesterol, fat and protein effects on serum cholesterol and atherosclerosis. In experiments conducted with the growing cockerel, dietary cholesterol consistently increased serum cholesterol and systolic blood pressure. Although high levels of soybean oil did not significantly affect serum cholesterol concentration, there were trends for increased blood pressure when diets contained high levels of soybean oil, corn oil or white grease.

Connective Tissue Components

Since this will be a discussion of connective tissue a description of these components, the fibroblast, collagen, elastin and ground substance, is in order.

The fibroblast, as discussed by Boucek et al. (1959) and Branwood (1963), is responsible for the production of ground substance, collagen fibers and steroid metabolism and synthesis. Fibroblasts apparently differentiate from the same primitive cells as smooth muscle cells and thus it has been postulated by Wissler (1968) that there is really only a multifunctional cell in the arterial media. Of extreme interest in atherosclerosis research is the finding that fibroblasts synthesize and secrete cholesterol and that this synthesis is greatly influenced by sex--the estrogens decreasing production and uptake of cholesterol by connective tissue; Boucek et al. (1959).

The fibrous components of connective tissue are collagen and elastin. Detailed discussions of collagen synthesis, structure, chemical and physical characteristics are covered in several reviews by Schmitt (1959), Gross (1959), Harrington and von Hippel (1961), Smiley and Ziff (1962), Lowther (1963), Ramachandran (1963) and Verzar (1964). Similar articles describing these properties of elastin are by Lansing (1959), Smiley and Ziff (1962), Partridge (1962) and Ayer (1964).

Elastin is characterized by its yellow color, extreme chemical inertness even at temperature up to 100°C, its amino

acid composition--low in acidic and basic amino acids and rich in monocarboxylic and monoamine amino acids and its lack of structure as measured by X-ray diffraction. Collagen on the other hand is readily converted to soluble gelatin upon autoclaving, contains a high proportion of proline and hydroxyproline (20%) and glycine (33%) and exhibits a characteristic periodicity upon X-ray analysis.

Extraction and purification of these two components is accomplished by a variety of methods, the simplest and most commonly used being that of Neuman and Logan (1950). Dry-defatted tissue is autoclaved several times for three hour periods at 15 psi to solubilize the collagen. Hydrolysis of the supernatant fraction and residue are then analyzed for hydroxyproline, the hydroxyproline content of collagen being 13.4% and of elastin ranging from 1.50% to 2.30% for different species.

Partridge (1962), however, states that further extraction with not dilute NaOH is necessary to remove all carbohydrates and tryptophan-containing protein. Since the hydroxyproline is variable in elastin tissues and regarded by some as collagen contaminant, gravimetric determination is recommended. In addition, tissues such as aorta contain appreciable amounts of calcium and, it is necessary to correct elastin for ash.

In studying the aging of collagen, several workers have isolated different collagen fractions based on the extraction medium. Jackson and Bentley (1960) sought to determine the

significance of the wide range of extractable collagen fractions and their position in fibrogenesis. Extraction of C^{14}-lysine labelled collagen from guinea pig skin and corrageenin granulomata was carried out in relation to time using neutral salt solutions of increasing ionic strength (0.14, 0.28, 0.45, 1.0 and 2.0 M NaCl), citrate buffer, pH 3.6 and gelatinization. At eight hours post injection the specific activity decreased with increasing ionic strength, but at 36 hours this was reversed except that the citrate buffer and gelatin fractions were still lowest. It was concluded that at any time there is a continuous spectrum of collagen aggregates with varying degrees of cross-linking, depending on the time from their synthesis. The varying extraction media remove different fractions of these aggregates depending on their disaggregating power, thus the collagen in 0.14 M NaCl fraction is considered the most recently synthesized.

Intimately associated with the cellular and fibrous components of connective tissue is a gel-like mixture called the ground substance which is composed of mucopolysaccharides and other carbohydrate and protein complexes. Mucopolysaccharides, according to the monograph by Brimacombe and Webber (1964), generally refers to heteroglycans containing uronic acid and hexosamine, in some cases sulfated, which are complexed in the native state with protein or peptide residues. Although accounting for less than 5% of the ground substance, mucopolysaccharides are responsible for its consistency. Biologically

they serve to bind water and act as tissue lubricants, as in the case for hyaluronic acid, and at the other extreme act as interfibrillar bridges as is postulated for the chondroitin sulfates, Muir (1964).

Arterial Wall Structure

The aorta is one of several arteries of interest in atherosclerosis research and also the aorta and pulmonary artery are the tissues which have been used extensively in arterial connective tissue studies. The thoracic aorta and pulmonary artery are both elastic type arteries as described by Partridge (1962), Bertelsen (1963) and Bloom and Fawcett (1962), in which three layers are distinguishable. The tunica intima is surfaced by an endothelium below which are thin elastic fibers, collagenous fibers, a number of fibroblasts and an abundance of ground substance. The interior layer or tunica media is the thickest layer and is bounded on either side by a rather poorly defined internal and external elastic membrane. Between these limiting membranes are 50-60 concentric elastic membranes which are described as clockwise and counter clockwise helices with occasional openings called fenestrations. Located between these membranes are the ground substance, collagen fibers, fibroblasts and smooth muscle cells. On the exterior is the tunica adventitia, a relatively thin layer, which cannot be sharply distinguished from the surrounding loose connective tissue.

The abdominal segment of the aorta changes to a muscular type of artery, again composed of three distinct layers. In this type of artery the internal and external elastic membranes are well defined. The media is composed almost exclusively of smooth muscle cells in concentric layers with thin elastic fibers between the two limiting membranes. The adventitia is generally thicker than the media and again blends into the surrounding connective tissue.

The nutrition of arterial tissue as described by Woerner (1959) is carried out by arteriols arising from the adventitial side and invading only the outer one-third of the media. The intima and inner one-third of media, receive their nutrition by diffusion from the lumen.

The aorta of the chicken is slightly different from that of man. Katz and Stamler (1953) indicate that the thoracic aorta contains slightly more elastic tissue than the corresponding segment of the human. The chick aorta changes to the muscular type in the abdominal region but lacks an internal elastic membrane as is in the human artery. Grollman et al. (1963) described the chicken aorta as resembling that of the human in the thoracic section but being composed of only two layers in the abdominal section. The inner two-thirds being circularly arranged muscle cells and the outer one-third being composed of elastic membranes. The intima in both segments of the chicken aorta appears similar to human intima.

Ayer (1964) described the role of various structural components previously discussed as providing controlled reversible deformability and a dampening mechanism for contraction and stretch. Burton (1954) indicates the function of smooth muscle in arterial walls is to change tension and this requires energy. Elastin provides an initial resistance to stretch without energy expenditure and collagen provides the final resistance again without energy expenditure.

Histological Observations in Aging and Atherosclerosis

Studies relating to humans

Numerous studies of arteries by histological and histochemical techniques have been reported. The previous general description of arterial structure was determined by these procedures.

The first notable change in the connective tissue of the human aorta from that previously described is fragmentation of the elastic tissue (Taylor, 1953; Bertelsen, 1961a; Bertelsen and Jensen, 1960; and Zugibe and Brown, 1960). This fraying and fragmentation, accompanied by reduplication, is noted first in the internal elastic membrane and begins to occur soon after birth. It has been demonstrated to occur and to increase in severity through all age groups studied from 0 to 90 years of age.

Accompanying this change in elastic tissue, numerous workers have observed changes in mucopolysaccharides. Mucopolysaccharides appear to be normal constituents of the arterial wall and are present to some degree throughout life. In the media, which is rich in elastic tissue, they appear to be evenly distributed in the fetal tissue and then their metachromic staining increases with aging up at least to the fifth decade (Banting and Banting, 1953; Taylor, 1953; and Bertelsen and Jensen, 1960). Taylor (1953) and Bertelsen and Jensen (1960) report that this mucopolysaccharide accumulation is intimately associated with the fragmentation of the elastic laminae of the media. After approximately the fifth decade this mucopolysaccharide staining of the media is relatively constant or diminishes due to increased fibrosis. Banting and Banting (1953) and Gresham and Howard (1961) report that maximum mucopolysaccharide production appears with the development of the fatty streak and continues with the development of the fibrous lesion of later life in the intima until it becomes acellular and hyaline.

Coronary and cerebral arteries from individuals ranging in age from fetal life to 70 years of age were examined and the results reported in two papers by Zugibe (1963) and Zugibe and Brown (1961). These results were compared with their observations of aorta tissue, Zugibe and Brown (1960). Elastic tissue changes were observed in all arteries although less frequently in the coronary arteries and at a later age in cerebral

arteries. No definite correlation could be made between the increased mucopolysaccharide staining also observed and the lipid deposition in the arteries or the elastic tissue fragmentation. Similar findings were reported by Laufer et al. (1962) for coronary arteries of 85 individuals of both sexes 30-35 years of age. Elastic fiber degeneration was the first alteration observed in atherogenesis and concomitantly large mucopolysaccharide deposits appeared in the intima. They could demonstrate no correlation between these two occurrences however.

Pulmonary artery tissue was studied by Bertelsen (1961a) along with aortic tissue and although structurally similar their aging processes are markedly different. Aging of pulmonary tissue is much less severe; little change in mucopolysaccharides is observed, there is less fragmentation of the elastic membranes and very little calcification of the media. This aging picture is the normal state however and in cases of pulmonary hypertension this artery more closely resembles the aorta in its age changes.

Another age and possibly atherosclerosis-related change is the calcification of the media of arteries. Bertelsen (1961a) found this began to occur very early in life in the case of the aorta and progressed in most cases very uniformly in the medial layer. He suggested that this was a normal calcification of the organic matrix in which the crystals are deposited in the chondroitin sulfate in close relation to the collagen fibrils.

However, in another study (Bertelsen, 1961b), of normal tissue and atherosclerotic plaques he found that the media below fibrous plaques always showed vigorous mineralization whereas lipoidal plaques were often seen overlying unmineralized media. It appeared that intimal alteration can be greatly accelerated by medial calcification.

Studies with experimental animals

The morphology of the spontaneous and induced atherosclerotic lesion in the chicken is described from early studies in some detail by Katz and Stamler (1953). The spontaneous lesions are elevated longitudinal ridgelike plaques restricted primarily to the lower abdominal aorta. They are described as a fibrous thickening of the intima and do not stain with Sudan IV nor is lipid demonstrable by histological examination. The cholesterol-induced lesion is described as most prevalent in the thoracic aorta and seen initially as a granularization of vessel surface and finally as elevated nodules, and raised longitudinal plaques. The abdominal non-lipid spontaneous lesion was reported to develop later but its severity is increased by cholesterol feeding. In addition, plaques similar to those of the thoracic segment appear. Histologically, these consist of an intact endothelium with underlying cells which stain for lipid as well as cholesterol. In the more advanced lesion the media is infiltrated and the elastic fibers split and later calcium can be demonstrated.

More recently Grollman et al. (1963) described various grades of the atherosclerotic lesions observed in chickens fed diets deficient in K, Cl, or cholesterol, or high in NaCl or squalene. As lesion severity increased, increased staining due to mucopolysaccharides in the intima was observed and lipid and cholesterol increased. In the most severe lesions the plaques were located deep in the intima, contained much lipid and mucopolysaccharides and in some cases calcium was present.

Rossi et al. (1965) made a comparative study of the serum lipids and the histochemical features of the arterial wall of several species of animals which differ in their susceptibility to experimental atherosclerosis. Species examined were the rat, mouse and hamster, which are resistant to cholesterol induced atherosclerosis, the guinea pig, dog, monkey and swine which are fairly susceptible and the rabbit, pigeon and chicken which are highly susceptible. Both males and females were examined. There appeared to be no correlation between any of the serum lipid components and atherosclerosis susceptibility. However, there was a constant parallelism between acid mucopolysaccharide concentration of the arterial wall and specie susceptibility to cholesterol atherosclerosis.

The feeding of 10% of several oils with added cholesterol was reported by Banerjee et al. (1965) to increase the lipid and mucopolysaccharides of the aortas in chickens 8-13 weeks of age. These changes, as well as intima proliferation and fragmentation or complete disappearance of the elastic tissue of

the intima and media, were most severe in the aorta arch region. The authors suggest that these elastic lesions coupled with the disturbances in mucopolysaccharide metabolism may alter the arterial permeability and result in atheroma formation.

Biochemical Observations in Aging and Atherosclerosis

In an attempt to clarify the histochemical observations concerning connective tissue changes and to put more quantitative units on these components, numerous reports of biochemical analyses are found in the literature.

Studies relating to humans

Elastin and collagen content of normal human aorta and pulmonary artery tissue as related to age was determined and reported in papers by Faber and Møller-Hou (1952), Lansing et al. (1950), Lansing (1954) and Bertelsen (1961b and 1962). Elastin was determined as the residue following treatment by autoclaving and NaOH digestion in these studies, and collagen usually was estimated from the tissue hydroxyproline content.

Faber and Møller-Hou (1952) in analyzing aortas from 85 individuals aged 20-70 years found that elastin decreased with age from 35% to 22% of the dry tissue weight while collagen increased from 20% to 30.5%. At any time in the normal human aorta collagen plus elastin amounted to 55% of the dry tissue weight of the vessel. In cases of hypertension the changes in

elastin and collagen are increased, especially in later years.

In the normal aorta media elastin content was reported by Lansing et al. (1950) to be relatively constant at 42% of the dry defatted weight from 20-100 years of age. Lansing concluded that the decreased elasticity observed with age is not due to gross decreases in elastin but perhaps changes in the elastin molecule. The pulmonary artery exhibited an increase in elastin from 31% during the first 3 decades to approximately 34-37% in the 7th and 8th decades. Collagen was measured separately on intima and media by hydroxyproline levels in the studies by Bertelsen (1961b and 1962). Medial hydroxyproline was relatively constant at 3.2% for both aorta and pulmonary arteries. In the aorta intima, however, an increase with age was found with the arteries from individuals below 40 years containing 3.36% while these over 40 years of age contain 4.64% expressed on a dry, defatted, calcium-free basis. Contrary to this, Kanabracki et al. (1960) using similar procedures on combined intima and media samples, and Smith (1965) in the analysis of lesion-free intima found that collagen did not change with age. Although slightly higher in children, Kanabracki stated that collagen accounted for about 20% of the aorta dry weight in all age groups whereas Smith calculated collagen to be approximately 24% of the protein in aorta intima.

Pernis and Clerici (1957) analyzed hyaline plaques and Noble et al. (1957) and Smith (1965) analyzed intimal samples in various stages of atherosclerosis. These workers noted an

increase in collagen constituents (hydroxyproline and glycine) in fatty streaks as well as advanced lesions of atherosclerosis. In calcified intimal plaques examined by Smith, collagen accounted for 60% of the protein.

Medial calcification occurring in aging has been described in histological examination and confirmed by several reports by biochemical analysis. Lansing et al. (1950) found that calcification of the media preceeded intimal plaque formation and elastic tissue breakdown. Although elastin content of the media did not change with age, calcium increased from 0.7% in the first decade to 6% of the dry, fat-free weight in the 5th decade. These workers felt that calcium deposition may play a significant role in atherosclerosis since deposition was more severe under plaque areas than in surrounding normal tissue. This concept is supported by the observation that calcification of the pulmonary artery seldom occurs to anything approaching this extent nor are the elastic tissue changes observed and atherosclerosis is entirely lacking. Kanabracki et al. (1960) reported similar calcium increases from 0.7% in children to 8.4% in the 60-69 year old group. In the female aorta, calcium was considerably lower up to 65 years of age and then increased sharply. This finding also correlates with the general lack of atherosclerotic changes in females until menopause.

Weissman and Weissman (1960) used X-ray diffraction analysis to study human aortic elastin calcification. Crystallites identified as hydroxyapatite and associated with the elastin

residue became increasingly prominent in aortae of older individuals. The X-ray diffraction pattern paralleled a decrease in nitrogen and an increase in calcium of the elastin residue with increasing age. The crystallite pattern was not observed for the collagen and polysaccharide fraction isolated from the aorta. Elastase treatment demonstrated hydroxyapatite association with elastin. The non-dialyzable protein of the supernatant solution of the digestion mixture gave the typical hydroxyapatite pattern.

Calcium as calcium phosphate and calcium carbonate was shown to accumulate with age in the aortae of Negroes and whites in further studies by Yu and Blumenthal (1963). The Ca/P ratio of the isolated aorta elastin approached that of a carbonate apatite during the second decade and remained fairly constant thereafter. X-ray diffraction analysis showed this elastin calcium to be in the form of carbonate apatite or a mixed carbonate-hydroxyapatite.

Elastin isolated from the media of older individuals or from calcified plaques exhibits an increased proportion of acidic amino acids compared to elastin of younger individuals and plaque-free aorta. Lansing et al. (1950), Weissman and Weismann (1960) and Yu and Blumenthal (1963) all suggested this increased acidity of elastin was responsible for its increased affinity for calcium.

Studies with experimental animals

Collagen and elastin were estimated in the aorta of normal male and female chickens, gonadectomized and oestradiol treated cockerels and testosterone treated hens by Cembrano et al. (1960). Males had significantly more aortic collagen than females, 35.7% vs. 29.5% and significantly more aortic elastin; 1.22% vs. 0.77% elastin hydroxyproline. Gonadectomy or oestradiol treatment decreased these components in males to the levels observed in females and similarly testosterone treatment of females resulted in increases to the level of the males.

Weiss and Fisher (1959) evaluated spontaneous atherosclerosis in 42 month-old-White Leghorn males, females, capons and poulards by several measures; plasma cholesterol, blood pressure, and aortic cholesterol and hydroxyproline. Hydroxyproline was higher in the abdominal than thoracic segment, the values for males being 6.8 and 5.0% of the dry weight respectively. Aortic cholesterol was highly correlated with plasma cholesterol for the various groups and blood pressure was highly correlated with aortic hydroxyproline. In evaluating the scores of the respective segments, plasma cholesterol and aorta cholesterol were more important for the thoracic segment; while for the abdominal segment, blood pressure and aortic hydroxyproline were more important.

Fisher and Griminger (1963) reported in a study of the changes in body composition in relation to aging that 80% dietary restriction did not significantly affect carcass

moisture, nitrogen or lipid. Hydroxyproline was also measured at various ages in several tissues including the aorta. Aorta hydroxyproline peaked at three months, being approximately 4.0% of the dry weight of the whole aorta. At the six- and twelve-month sampling periods, the full-fed birds had a significantly lower hydroxyproline concentration in the abdominal aorta, 6.8% vs. 7.8%. This was attributed to the increased fat content of this segment for the full-fed birds. At these sampling periods the thoracic hydroxyproline concentration was approximately 4.0%. A decline was noted at 38 weeks from these values to 3.0% and 5.2% of the dry defatted weight of the respective segments.

Gan et al. (1967), following a fairly extensive fractionation procedure, estimated the changes in acid mucopolysaccharides, glycoproteins, collagen and elastin in male and female White Leghorn chickens ranging in age from 3 to 36 months. Collagen was higher in the aorta segment and elastin was higher in the thoracic segment. Collagen increased and elastin decreased with age in both sexes and in both aorta segments. In males from 3 to 36 months, the collagen increase was from 34 to 53 for the thoracic segment and 49 to 72 for abdominal segment of the aorta, expressed as mcg hydroxyproline per mg lipid-free dry tissue. Values for elastin in the male were reported as decreases from 16 to 11 and 8.8 to 4.5 for the respective segments, again expressed as mcg hydroxyproline per mg lipid

free dry tissue. Total hydroxyproline showed a linear increase with age in the abdominal segment; however, this was not evident until after 24 months of age for the thoracic segment. Total non-elastin protein was higher for the abdominal segment than for the thoracic segment (approximately 500 vs. 400 mcg/mg lipid free dry weight) and showed a linear and quadratic increase with age for the male and female respectively. The acid mucopolysaccharides were found to be higher in the thoracic segment and to decrease with age for both sexes and segments. Glycoproteins significantly increased with age in both segments and sexes and were higher in abdominal segments than in the thoracic aorta. Both total and free cholesterol in the aorta of both sexes showed a tendency to increase with age. This amounted to a 4-5 fold increase for the abdominal segment and was linear for the male and quadratic for the female. Thoracic cholesterol changes were much less marked. Plasma cholesterol (total and free) decreased with age for the male; however, plasma-free cholesterol in females increased with age.

Rather marked differences in the distribution of collagen and elastin in different regions of the aorta were reported by Grant (1967). Dry fat-free segments of the arch, thoracic and upper and lower abdominal regions of the aorta from pigs, sheep, goats and man were analyzed. In each specie, the elastin content decreased and collagen content increased as the vessel was descended from arch to lower abdominal region. The most marked change was in the pig where elastin decreased from

51 to 9% with a corresponding decrease in the elastin/collagen ratio from 3.2 to 0.2. The corresponding values for elastin in the sheep and goat aorta were 42 to 27%, and 44 to 33% with a change in the elastin/collagen ratios of 2.56 to 0.91 and 2.10 to 0.61 respectively. Human aorta elastin content showed the least variation with a decrease from 38 to 21% and the corresponding elastin/collagen ratios were 1.63 to 0.77. Total elastin hydroxyproline content showed a consistent increase for all species as the aorta was descended. Total elastin plus collagen was relatively constant along the aorta for sheep, 60%, and goat, 65%, with a slight decrease in the human, 60 to 50%, and pig, 68 to 50% as the aorta was descended. Hexosamine and uronic acid did not show marked variation with site and bore no relationship to collagen or elastin content.

Harkness et al. (1957) reported similar results in a study of collagen and elastin contents of the arterial walls of the dog. Although the total elastin and collagen formed about 50% of the aorta dry weight, there was a rather abrupt change in proportion as the aorta was descended. They found about twice as much elastin as collagen in the thoracic segment and the opposite was true for the abdominal region. This change, they reported, took place over a distance of approximately 5 cm.

Using male and female rats aged three to five weeks, eight months, and two years, McGavack and Kao (1960) studied the influence of age and sex on nonscleroprotein, soluble collagen,

insoluble collagen and elastin of several tissues. Nonscleroprotein decreased with age in the tendons and dermal tissue of both male and female rats. Insoluble collagen continued to increase with age in the tail tendon, aorta and skin of females up to two years of age, while in males a plateau was reached for these tissues at eight months of age. The percentage of soluble collagen of the total collagen in the skin and tendon of females decreased gradually with age while soluble collagen in the aorta decreased rapidly. In the male, soluble collagen decreased rapidly in tendon, aorta and skin. More elastin was found in males than females at eight months of age and tail tendon elastin increased for both sexes with age.

Synthesis and turnover of connective tissue protein was investigated and reported in two papers by Kao et al. (1961a and 1961b). In the first study, C^{14}-lysine-injected five-week-old rats were sacrificed at intervals of one hour to 90 days post injection. Nonscleroprotein, soluble collagen, insoluble collagen and elastin were isolated from several tissues and turnover and synthesis estimated from the specific activities. Specific activities of the several fractions were reported in decreasing magnitude to be nonscleroprotein, soluble collagen insoluble collagen and elastin. Synthesis was observed, in decreasing magnitude to occur in the uterus, skin, aorta and tendon and turnover was observed, in decreasing magnitude, to occur in the uterus, skin, tendon and aorta. In the second paper, rats five weeks, eight months and two years of age were

injected with C^{14}-lysine and the specific activities of the same tissues and fractions measured as in the previous experiment at intervals up to 40 days past injection. With the exception of uterine tissue, insoluble collagen and elastin were synthesized at significantly higher rates by five-week-old than eight month and two-year-old rats. The uterus was the only tissue which showed appreciable insoluble collagen synthesis and no detectable synthesis was found for this component in tendon tissue at two years of age. Collagen turnover was low in all animals even at five weeks of age.

Gross (1958) measured the neutral salt-citrate buffer and dilute acetic acid-extractable collagen in relation to rate of growth in suckling guinea pigs. Neutral salt-extractable collagen (0.45 M NaCl) amounted to about 10% of the total collagen of the dermis in growing guinea pigs and this was equivalent to about a one to two days increment in collagen growth. Static weight maintained by limited caloric intake reduced non-scleroprotein to very low levels and twelve days of steady weight gain was required to return this fraction to normal. Neutral salt collagen of the dermis varied directly with growth rate and was greatly diminished after short periods of restricted caloric intake. Two days of fasting reduced this fraction by one-half. Citrate buffer extraction of the neutral salt residue removed 40% more collagen from the actively growing animals than from those fasted for two days. Subsequent extraction of the residues with dilute acetic acid however

equalized the total amount of collagen extracted at acid pH from the two groups.

Soluble and insoluble collagen of the aorta of rats fed diets rich in sucrose, glucose, fructose or starch was measured in a study by Cohen and Shoshan (1968). They observed that 0.1M acetic acid soluble collagen was increased and the insoluble/soluble collagen ratio was reduced in rats fed the several sugar diets compared to those fed the starch diet. Lowering the protein from 18% to 11% increased the soluble collagen in all groups and also increased the observed differences between the starch and sugar-fed groups.

EXPERIMENTAL PROCEDURE

General Management and Dietary Treatments

Male chickens of the Hubbard commercial broiler strain were used for the two experiments reported here. The chicks were hatched at the Iowa State University Poultry Research Center and vent-sexed at day of age. All birds were reared to four weeks of age in electrically heated wire-floored starter batteries, then transferred to unheated wire-floored grower batteries until eight weeks of age. Water and a standard Iowa State University chick starter ration formulated to contain 20% protein and 1250 metabolizable Calories per pound were available ad libitum until the experiments were started at eight weeks of age.

Experiment 709

At eight weeks of age, 90 birds were selected, wing-banded and transferred to individual wire-floored cages housed in three environmental chambers. Temperature in the chambers was maintained at 70° ± 5°F, and lights were adjusted to provide a 12 hour day length. Water was available ad libitum and birds were individually fed each day.

Semipurified experimental diets[1] shown in Table 1 were

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[1]Diet designations for Experiment 709 to be used in the text and all following tables will give the percent protein, 10 or 20, followed by the energy concentration in metabolizable energy Calories per kilogram of diet, 3300 or 2200, with the abbreviation Chol. indicating those diets containing added cholesterol. For example; the 20% protein, 3300 metabolizable energy Calories per kilogram of diet will be designated 20-3300 and the 10% protein, 2200 metabolizable Calories per kilogram of diet with 1% cholesterol will be referred to as 10-2200 + Chol.

Table 1. Composition of diets for Experiment 709

	% of diet				
	20-3300	10-2200	10-2200 + Chol.	10-3300	10-3300 + Chol.
Isolated soybean protein	23.0	11.2	11.2	11.2	11.2
Glucose	64.0	43.0	43.0	77.0	77.0
Alphacel	3.4	6.2	5.2	2.2	1.2
Soybean oil	4.0	4.0	4.0	4.0	4.0
Premix 709[a]	1.5	1.5	1.5	1.5	1.5
Mineral mix[b]	4.1	4.1	4.1	4.1	4.1
Cholesterol	----	----	1.0	----	1.0
Total	100.0	70.0	70.0	100.0	100.0
Calculated analysis[c]					
Protein, %	20	10	10	10	10
Energy, M.E. Cal./kg	3300	2200	2200	3300	3300

[a]Premix 709 provides per kilogram of diet; vitamin A 8800 IU; vitamin D 880 ICU; vitamin E 44 IU; vitamin K 4.4 mg; riboflavin 11 mg; pantothenic acid 16.50 mg; niacin 16.50 mg; pyridoxine 4.4 mg; thiamine 2.64 mg; choline 1935 mg; folacin 1.54 mg; vitamin B_{12} 1.32 mcg; biotin 132 mcg; inositol 1100 mg; p-aminobenzoic acid 110 mg and methionine 0.25% glycine 0.25% and santoquin 0.013%.

[b]Mineral mix provides per kilogram of diet: calcium 10.2 gm; phosphorous 5.8 gm; sodium 1.6 gm; potassium 1.9 gm; manganese 51 mg; iron 51 mg; copper 5.1 mg, and zinc 51 mg.

[c]Calculated analysis for diets II and III is expressed on a 100% basis since these diets were fed at a 70% level.

formulated and fed in such a manner to provide various combinations of 50% restriction of protein intake and a 33% restriction of energy intake on a body weight basis with and without added cholesterol compared to the basal diet. Diet 20-3300 was fed ad libitum. Birds receiving Diets 10-2200 and 10-2200 + Chol. were fed daily, on a body weight basis, amounts corresponding to 70% of the consumption of the birds fed Diet 20-3300 so that it was not necessary to incorporate excessive amounts of the non-nutritive fiber filler into these diets. Diets 10-3300 and 10-3300 + Chol. were fed daily, on a body weight basis, amounts corresponding to 100% of consumption of birds receiving Diet 20-3300. Body weight and calculation of the feed allotments based on these individual body weights were made weekly. It was hoped this feeding regime would result in accurate dietary restriction; however to maintain body weights of some individual birds it was necessary to set a lower limit on the feed restriction at nine grams of protein per bird per day.

In addition to the 90 birds used in the feeding trial eight similar birds were sacrificed at eight weeks of age for an initial collection of aorta and liver tissues. Systolic blood pressure was measured and blood collections made on the experimental birds at age intervals of 8, 12, 16, 20, 24 and 32 weeks. At 16, 24 and 32 weeks of age six birds per treatment were sacrificed and aorta and liver tissues retained for later analysis.

Experiment 727

Eighty birds for this experiment were selected on the basis of uniform body weight at eight weeks of age, wing-banded and individually caged in 10" by 16" wire laying cages. These cages were in a windowless building at the Poultry Research Center which had supplemental heat and forced air ventilation such that temperature was maintained between 45°F and 85°F. Water was available ad libitum from drop waterers and the experimental diets were available ad libitum from individual feeders.

The dietary treatments in this experiment involved dietary protein, proportion of total calories from soybean oil, and dietary cholesterol in a 2 x 2 x 2 factorial arrangement. These diets[1] were formulated as shown in Table 2. Body weights and feed consumption were determined at two week intervals.

In addition to the 80 birds used in the feeding trial, six similar birds were sacrificed at eight weeks of age for an initial collection of aorta and liver tissues. Blood collections were made at intervals corresponding to 8, 12, 16, 20, 24, 28 and 30 weeks of age. At 16, 24 and 30 weeks of age

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[1]Diet designations for Experiment 727 to be used in the text and all following tables will give the percent protein, 10 or 20, followed by the percentage of total metabolizable energy Calories provided by soybean oil with the abbreviation Chol. indicating those diets containing added cholesterol. For example, the 10% protein diet with 40% of its calories from soybean oil will be referred to as 10-40 and the 20% protein diet with 10% of its calories from soybean oil and containing 1% added cholesterol will be referred to as 20-10-Chol.

Table 2. Composition of diets for Experiment 727

	% of diet[a]			
	10-10	10-40	20-10	20-40
Soybean meal	20.0	20.0	40.0	40.0
Glucose	62.6	36.7	47.5	12.1
Soybean oil	3.0	12.1	3.0	21.5
Solka floc	8.5	25.3	3.6	20.5
Premix 727[b]	1.0	1.0	1.0	1.0
Dicalcium phosphate[c]	3.6	3.6	3.6	3.6
Salt and trace mineral mix	1.3	1.3	1.3	1.3
Total	100.0	100.0	100.0	100.0
Calculated analysis:				
Protein	10	10	20	20
Energy, M.E. Cal./kg	2770	2770	2770	2770
% of calories supplied by soybean oil	10	40	10	40

[a]A corresponding diet in each case contained 1% added cholesterol.

[b]Premix 727 provides per kilogram of diet: vitamin A 10,000 IU; vitamin D 1,000 ICU; vitamin E 20 IU; vitamin K 2 mg; riboflavin 10 mg; pantothenic acid 30 mg; niacin 30 mg; thiamine 30 mg; pyridoxine 8 mg; folacin 2 mg; vitamin B_{12} 20 mcg; biotin 100 mcg; choline 800 mg; inositol 100 mg; p-aminobenzoic acid 30 mg; and methionine 0.25%; glycine 0.25%; and santoquin 0.013%.

[c]Salt and trace mineral mix provides per kilogram of diet; sodium 1.6 gm; manganese 51 mg; iron 51 mg; copper 5.1 mg; and zinc 51 mg.

three or four birds per treatment were sacrificed and aorta and liver tissues retained for later analysis.

Blood Pressure Measurement and Tissue Collection

Systolic blood pressure was measured by the indirect method similar to that described by Sturkie et al. (1957). A detailed description of the equipment and procedure used is given by Speers (1965).

Blood samples from each bird, amounting to approximately 10 ml, were collected by puncturing the wing vein with a sharp scalpel and allowing the blood to drip into a conical centrifuge tube. The blood was allowed to clot, then centrifuged and the serum decanted into a labelled screw-cap vial. The serum samples were then frozen and held for later analysis.

At the previously mentioned intervals, selected birds were sacrificed by cutting the jugular vein and carotid arteries. At necropsy any abnormalities were noted and the liver was removed, placed in a plastic bag and frozen for later analysis. The entire aorta from the junction with the heart to just past the iliac bifurcations was removed, cleaned of adhering connective tissue, placed in a plastic bag and frozen for later analysis.

After the experiment was completed and all aorta tissues collected they were thawed and opened longitudinally for examination and scoring. Thoracic and abdominal segments were scored separately, without staining, by gross visual examination

according to a subjective scale of one through four. A score of one indicated no visible abnormality, lipid deposition or plaque thickening, while a score of four indicated grossly visible lipid deposition or fibrous plaque formation or both.

Chemical Procedures

Serum cholesterol determination

Total serum cholesterol was determined using a Technicon Autoanalyzer according to the Technicon N-24 Methodology (Technicon Instruments Corp. Chauncey, New York). This method is based on reaction of a concentrated sulfuric acid ferric chloride-acetic acid color reagent with steroids having the 5-ene, 3-beta-ol grouping.

Liver lipid determination

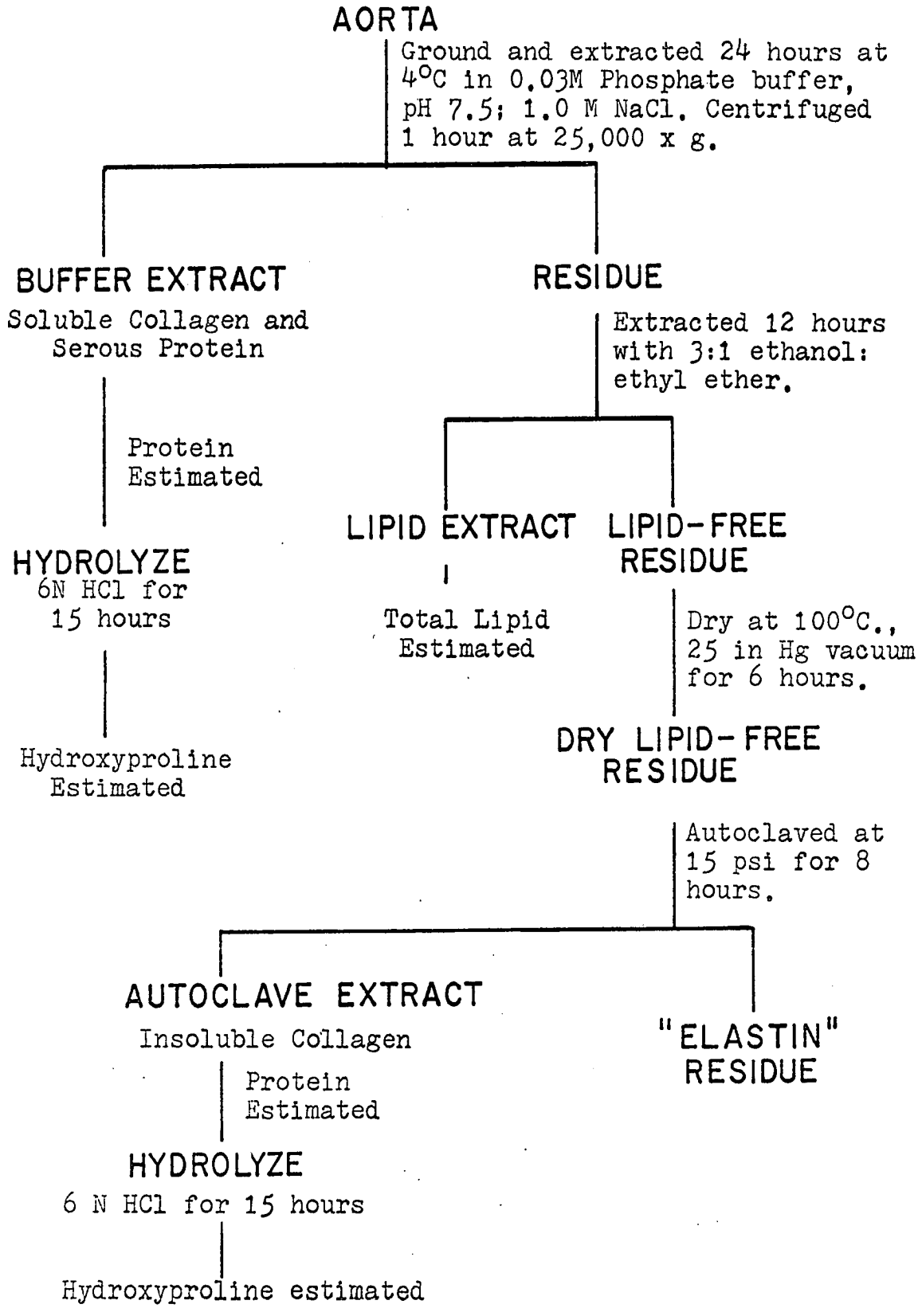
Total liver lipid was determined on duplicate dried samples using a Goldfisch extractor. Initially the entire liver was placed in a drying oven at 100°C for 24 hours. The samples were further dried for 12 hours at 100°C under 25 inches Hg vacuum. After reweighing the pans, the dried samples were ground to a fine powder in an Oster blender. Samples weighing 1-2 grams were then extracted for six hours on high heat with a solvent mixture consisting of ethyl ether, Skelly B and ethyl alcohol (5:5:1). Total lipid was determined directly by evaporating the solvent from the previously tared extraction beaker and reweighing. Total lipid was expressed as a percent of the dry weight.

Aorta analysis

A fractionation scheme similar to that of Weissman et al. (1963) was utilized for the estimation of neutral salt-soluble collagen, total lipid, insoluble collagen and elastin of the separate aorta segments. The fractionation scheme is shown in Figure 1. After the initial extraction, the isolation of collagen and elastin is essentially the method of Neuman and Logan (1950).

For extraction of neutral salt-soluble collagen and serous proteins, the separate aorta segment samples were thawed, blotted dry, a wet weight taken and the samples were then minced with a dissecting scissors. The minced tissue was then transferred to a 15 ml Potter Elvehjem tissue grinder along with approximately 5 ml of phosphate buffer (0.03M phosphate buffer, pH 7.5, 1.0M in NaCl). During grinding the tissue grinder and sample were cooled by submerging in ice water. The finely homogenized tissue was transferred to a 50 ml centrifuge tube with three or four 1 ml buffer washings and a $\frac{1}{2}$ inch magnetic stirring bar was added. The tubes were placed on a magnetic stirring motor in the cold room at $4^{\circ}C$ and extraction continued for 24 hours. Following buffer extraction the residue remaining was sedimented by centrifugation for one hour at 15,000 rpm (25,000 x g) in an International HR-1 refrigerated centrifuge equipped with a No. 856 angle head. The supernatant was decanted into a 10 ml volumetric flask and diluted to volume.

Figure 1. Fractionation scheme used in aorta analysis


AORTA
Ground and extracted 24 hours at 4°C in 0.03M Phosphate buffer, pH 7.5; 1.0 M NaCl. Centrifuged 1 hour at 25,000 x g.
BUFFER EXTRACT
Soluble Collagen and Serous Protein
Protein Estimated
HYDROLYZE
6N HCl for 15 hours
Hydroxyproline Estimated
RESIDUE
Extracted 12 hours with 3:1 ethanol: ethyl ether.
LIPID EXTRACT
Total Lipid Estimated
LIPID-FREE RESIDUE
Dry at 100°C., 25 in Hg vacuum for 6 hours.
DRY LIPID-FREE RESIDUE
Autoclaved at 15 psi for 8 hours.
AUTOCLAVE EXTRACT
Insoluble Collagen
Protein Estimated
HYDROLYZE
6 N HCl for 15 hours
Hydroxyproline estimated
"ELASTIN" RESIDUE

Total lipid was extracted from the buffer extract residue by resuspending the residue in 10 ml of Bloors reagent (3:1, ethanol: ethyl ether). Extraction was continued for 12 hours in the cold room at 4°C with two changes of Bloors reagents. When the reagent change was made and at the end of the lipid extraction period the residue was concentrated by centrifugation at 15,000 rpm (25,000 x g) for 20 minutes. The supernatant was decanted into a 25 ml volumetric flask and diluted to volume with additional solvent.

After lipid extraction, the buffer extracted, fat-free residue was quantitatively transferred with several water washings to a previously weighed aluminum pan and dried to constant weight. Initial drying was done in a drying oven at 60°-70°C and after most of the water was removed, the drying was continued for six hours in a vacuum oven at 95°-100°C under 25 inches Hg vacuum. The pan and residue was reweighed and extracted fat-free dry weight was calculated.

A portion, 30-100 mg, of dried, extracted fat-free residue was then transferred to 15 ml polyethylene centrifuge tube and 4-5 ml of distilled water added. The samples were then autoclaved at a pressure of 15 psi for four hours. The residue was then concentrated by centrifugation at 10,000 rpm (12,000 x g) and the supernatant decanted into a 25 ml volumetric flask. The residue was then resuspended in 4 ml distilled water, these steps repeated, and the second supernatant added to the same flask which was then diluted to volume.

Elastin was estimated gravimetrically by quantitatively transferring the autoclaved residue to a previously weighed aluminum pan. This was then dried to constant weight as described previously and reweighed.

After the several extracts were prepared for a group of samples the following determinations were made on these extracts. Total lipid was estimated gravimetrically by transferring duplicate 10 ml aliquots of the 25 ml lipid extract into each of two previously weighed aluminum dishes. The solvent was evaporated at 100°C in a drying oven and pan and residue reweighed. Protein was estimated in both the buffer extract and autoclave extract using the Folin phenol reagent according to the procedure described by Lowry et al. (1951). Soluble collagen and insoluble collagen measured as hydroxyproline, were estimated respectively in the buffer extract and autoclave extract using the modified method of Prockop and Udenfriend (1960). Aliquots of the extracts were placed in 150 mm screw cap culture tubes fitted with teflon lined caps, flushed with nitrogen and hydrolyzed with 6 N HCl. Hydrolysis was carried out for 15 hours in an autoclave at a pressure of 15 psi. The procedure was modified essentially by reducing all volumes by 50% so 125 mm screw cap culture tubes could be used in a clinical centrifuge.

Hydroxyproline values were converted to collagen by multiplying by the factor 7.46. This is the factor determined by Neuman and Logan (1950) since collagen preparations from

several mammalian and avian sources were found to contain 13.4 ± 0.24% hydroxyproline.

In the tables to follow, lipid collagen, elastin and protein are expressed on a fat-free dry weight basis. Since it was necessary to determine the dry weight after part of the material had been removed in the buffer extract and the lipid extracted, this is a calculated value. To the weight of the buffer-extracted, fat-free dry residue was added back the estimated weight of the total protein removed in the buffer extract. This quantity was then taken as the fat-free dry weight and all constituents expressed as a percentage or proportion of this.

Statistical Analysis

Experiment 709 was conducted in a randomized complete block design, blocking on body weight and chamber location. Due to excessive mortality, resulting in missing values, this experiment was analyzed as a completely random design. Experiment 727 was conducted and analyzed in a completely random design with the treatments arranged factorially.

Analysis was conducted by the Iowa State University Computation Center. A general least squares analysis of variance in regression format was used for the aorta score, aorta tissue analysis and liver lipid data due to disproportionate replication. Data on these variables collected at the initiation of the experiments (eight weeks of age) could not be included in the model for analysis, but is presented in the following

tables for comparison.

Data collected on a continuous basis from the same birds, serum cholesterol and blood pressure, were analyzed as a split plot with treatments as the main plot and periods or weeks as the sub-plot.

Main effect sums of squares in Experiment 709 were partitioned by use of orthogonal planned comparisons. The coefficients of these comparisons are shown in Table 23 of the Appendix. Similarly, the linear and quadratic components for weeks were partitioned by the use of orthogonal polynomials. The main effect and interaction sums of squares will not sum to the treatment sums of squares in most cases because of unequal replication.

Correlation coefficients were calculated within treatments for all data on aorta scores, aorta analysis, terminal serum cholesterol and terminal blood pressure.

RESULTS

Experiment 709

The purpose of Experiment 709 was to study the effect of various combinations of 50% protein intake restriction and a 33% energy intake restriction, expressed on a body weight basis, with and without dietary cholesterol on several physiological factors relating to atherosclerosis and the connective tissue components of the avian aorta. Weight gains and the effectiveness of the restriction program for protein and energy are shown in Table 3.

Only during the first period, 8-16 weeks of age, was the desired level of restriction approached. During this period birds fed 10-2200 diets were allowed to consume, on a body weight basis, 54% as much protein and 72% as much energy as the birds fed the 20-3300 basal diet. Protein was restricted to 49% of the basal with essentially the same caloric intake, both expressed as per kilogram body weight, for the birds fed the 10-3300 diets. A lower limit of not less than nine grams of protein and 200 metabolizable energy Calories or 300 metabolizable energy Calories per day for the birds fed the 10-2200 and 10-3300 diets respectively had to be imposed soon after the experiment started to prevent continuous weight loss. This limitation along with the very reduced growth rate of the birds fed the 10-2200 resulted in the protein intake for this group of 75% rather than the desired 50% compared to the basal group

Table 3. Effect of dietary protein and energy restriction, with and without cholesterol, on weight gain and protein and energy consumption of cockerels. Experiment 709

Treatment	Age, weeks			
	8-16	16-24	24-32	8-32
20-3300				
Weight gain (gm)	2164	496	273	2933
Protein/day (gm)	25.21	23.37	23.45	24.01
Prot./day/kg body wt. (gm)	9.67	5.94	5.39	6.83
M.E. Cal./day	417	397	392	403
M.E. Cal./day/kg body wt.	160	101	90	117
10-2200				
Weight gain (gm)	463	226	246	935
Protein/day (gm)	9.26	9.34	9.89	9.50
Prot./day/kg body wt. (gm)	5.22	4.44	4.33	4.66
M.E. Cal./day	204	206	217	209
M.E. Cal./day/kg body wt.	115	98	95	103
10-2200 + Chol.[a]				
Weight gain (gm)	455	213	217	885
Protein/day (gm)	9.23	9.12	9.50	9.28
Prot./day/kg body wt. (gm)	5.24	4.35	3.98	4.52
M.E. Cal./day	203	197	210	203
M.E. Cal./day/kg body wt.	115	94	88	99
10-3300				
Weight gain (gm)	1095	647	518	2260
Protein/day (gm)	11.20	9.58	10.18	10.32
Prot./day/kg body wt. (gm)	4.74	3.26	2.92	3.64
M.E. Cal./day	371	317	335	341
M.E. Cal./day/kg body wt.	157	108	96	120
10-3300 + Chol.[a]				
Weight gain (gm)	1212	644	338	2194
Protein/day (gm)	10.12	9.57	9.99	9.90
Prot./day/kg body wt. (gm)	4.70	3.14	2.85	3.56
M.E. Cal./day	336	320	330	329
M.E. Cal./day/kg body wt.	156	105	94	118

[a] 1% cholesterol.

during the next two periods. Energy intake, expressed on a body weight basis, did not differ for the 20-3300 and the 10-2200 groups. Protein intake of the birds fed the 10-3300 diets was approximately 53% of the basal group during the periods from 16-24 and 24-32 weeks of age with essentially similar caloric intake.

Although the dietary restrictions calculated on a body weight basis were not realized in all instances, an absolute restriction of protein and energy on a per day basis was obtained. This amounted to 39% and 42% daily protein intake of the birds fed the 10-2200 and 10-3300 diets respectively compared to the 20-3300 basal group. Energy intake, on a daily basis, for these groups was 52% and 83% compared to the birds fed the 20-3300 diets over the 8-32 week experimental period.

Weight gains were much more severely retarded by energy restriction than by protein restriction, and were not significantly influenced by dietary cholesterol. Over the 8-32 week experimental period the birds fed the 10-2200 diets gained only 30% as much weight as the basal group, while those birds fed the 10-3300 diets gained 75% as much weight as the basal group. For all groups one-half or more of the total growth occurred in the first eight weeks of the experiment. For the restricted groups in the subsequent periods, growth was only approximately 50% that of the first eight weeks because the intake restriction became more severe as the birds grew larger. Weight gains for the birds fed the 20-3300 basal diet also were much less

after the first eight weeks on experiment because they had essentially reached mature body size by 16 weeks of age.

Serum cholesterol concentration was rapidly and significantly ($P \leq 0.01$) increased by the addition of 1% cholesterol to the diets as shown in Figure 2 and Table 4. A significant ($P \leq 0.01$) energy x cholesterol interaction is quite evident in Figure 2, with the birds fed the higher energy diet exhibiting twice the serum cholesterol level compared to those fed the lower energy diet. In the absence of added dietary cholesterol, neither energy nor protein intake significantly influenced serum cholesterol concentration although significant effects are indicated due to the way the comparisons were made. There may have been a trend for lower serum cholesterol with a higher protein intake. The rapid rise in serum cholesterol level of the cholesterol-fed birds compared to the others resulted in a highly significant treatment x age interaction as would be anticipated.

Systolic blood pressure (Figure 3 and Table 4) was significantly ($P \leq 0.01$) increased by either increased protein or increased energy. Birds with an increased protein intake or a decreased energy intake exhibited a significant ($P \leq 0.01$) increase or decrease in systolic blood pressure respectively. The greatest influence was due to protein with birds fed the 20-3300 diets showing the highest systolic blood pressure and those fed the 10-3300 diets intermediate compared with those

Figure 2. Effect of protein and energy restriction and dietary cholesterol on serum cholesterol concentration. Experiment 709

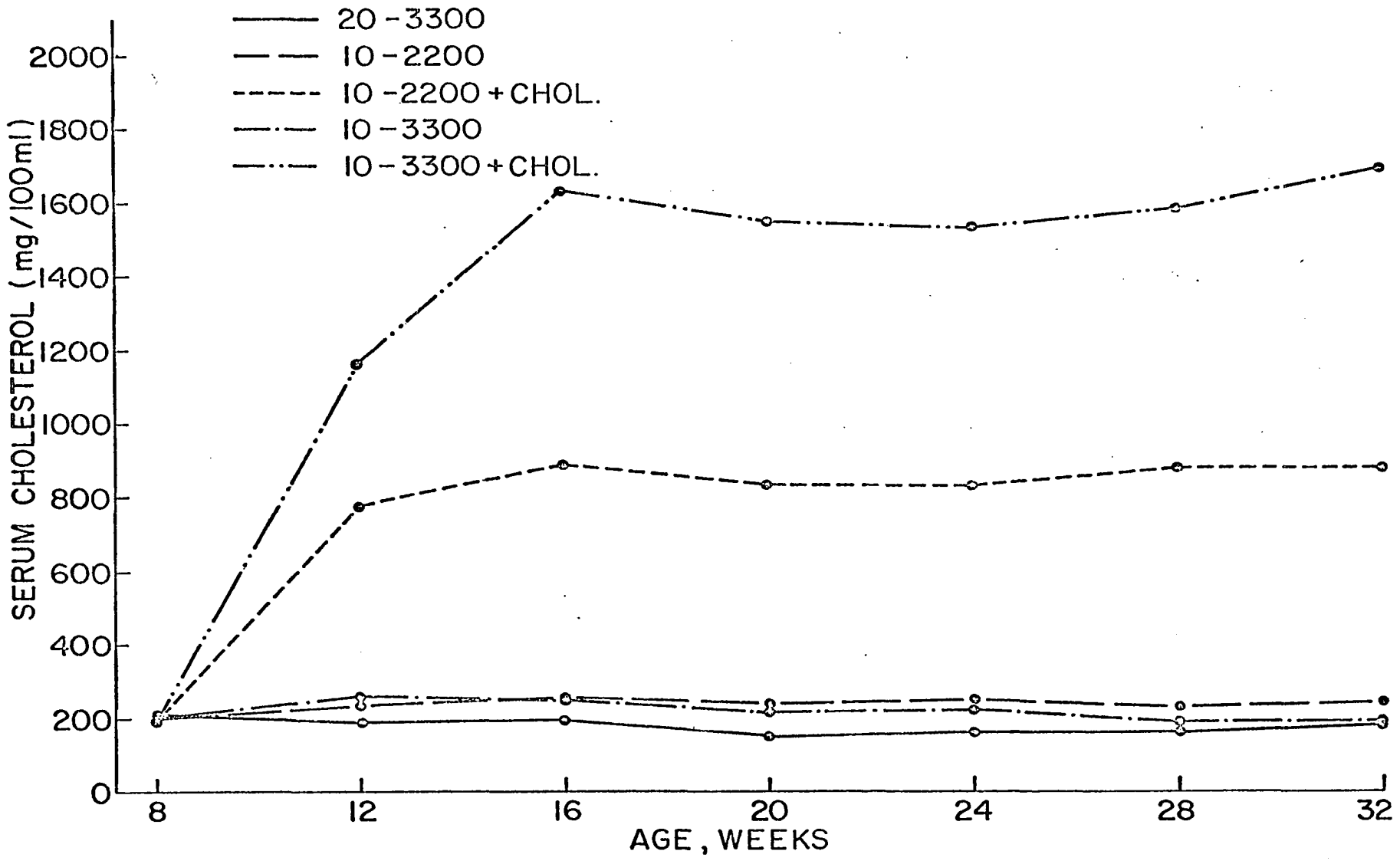

20 - 3300
10 - 2200
10 - 2200 + CHOL.
10 - 3300
10 - 3300 + CHOL.
SERUM CHOLESTEROL (mg/100ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
8
12
16
20
24
28
32
AGE, WEEKS

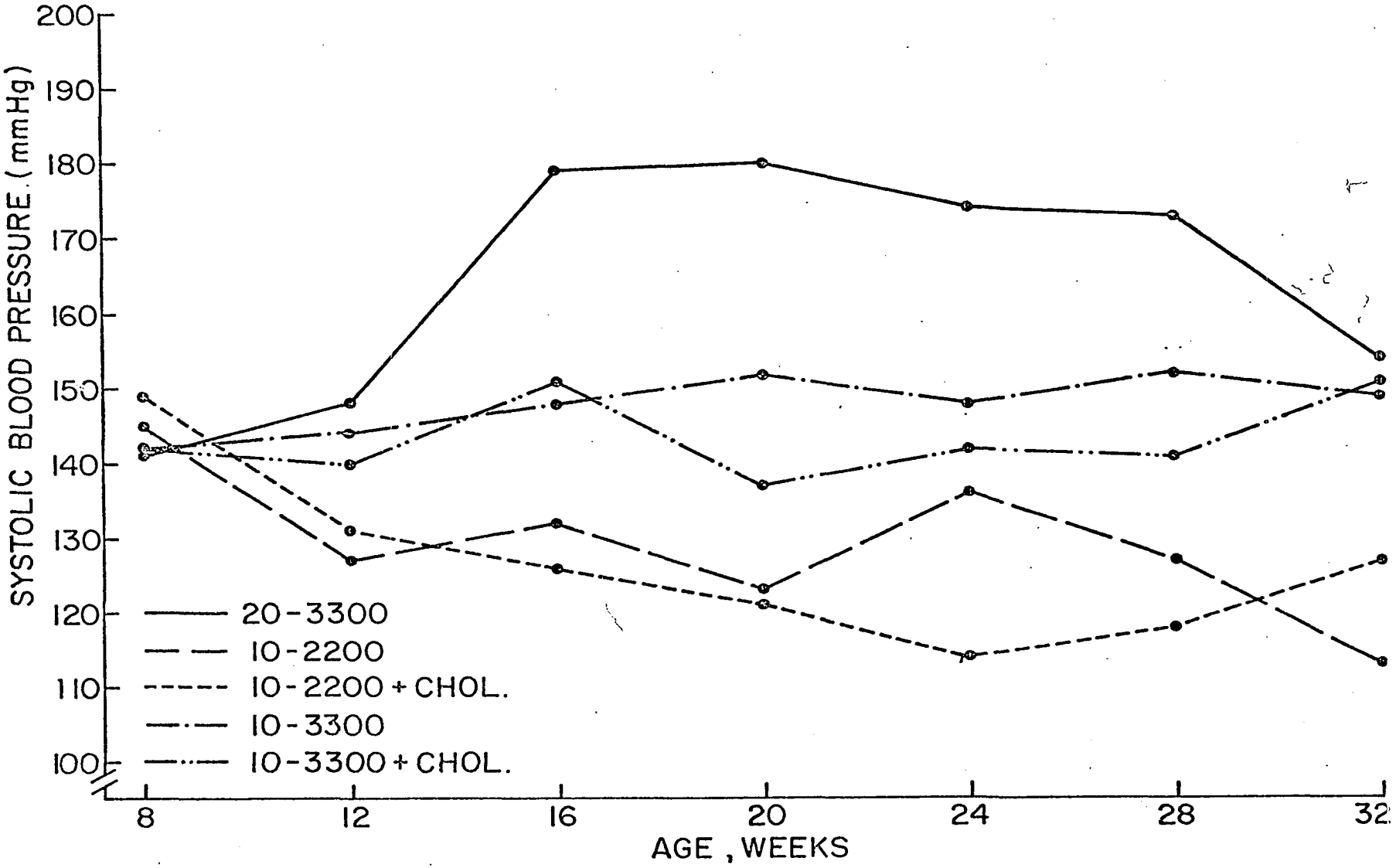
Table 4. Effect of dietary protein and energy restriction with and without dietary cholesterol on cockerels. Experiment 709

Treatment	Average data over 8-32 weeks				
	Thoracic aorta score	Abdominal aorta score	Systolic blood pressure (mm Hg)	Serum cholesterol mg/100 ml	Liver[a] lipid %
20-3300	1.00	1.92	164	178	13.85
10-2200	1.00	1.31	129	239	9.21
10-2200 + Chol.[b]	1.27	2.73	127	756	22.09
10-3300	1.00	2.12	148	219	12.94
10-3300 + Chol.	1.73	2.60	143	1336	32.29

[a]Dry weight basis.

[b]1% cholesterol.

Figure 3. Effect of protein and energy restriction and dietary cholesterol on systolic blood pressure. Experiment 709


SYSTOLIC BLOOD PRESSURE (mmHg)
200
190
180
170
160
150
140
130
120
110
100
20-3300
10-2200
10-2200 + CHOL.
10-3300
10-3300 + CHOL.
8
12
16
20
24
28
32
AGE, WEEKS

birds consuming the 10-2200 diets. Due to the various treatment effects again a highly significant treatment x age interaction was observed.

Total liver lipid concentration was not significantly influenced by age as seen by the age averages of the data in Table 4. The statistical analysis of the treatment effects however indicated significant ($P \leq 0.01$) influences attributed to all the dietary treatments and a highly significant energy x cholesterol interaction. The energy x cholesterol interaction is clearly evident in Figure 4 showing that dietary cholesterol increases liver lipid more in birds fed the higher energy diets with restricted protein than in those fed the restricted protein, lower energy diets. Because of the energy x cholesterol interaction and the manner in which the protein and energy main effect comparisons were made, interpretation of these main effects is more difficult. A true protein effect is doubtful when the liver lipid concentration of the birds fed the 20-3300 and 10-3300 diets are compared. Similarly, dietary cholesterol influenced the energy effect significance but comparison of the liver lipid concentration of birds fed 10-2200 and 10-3300 diets seems to indicate that liver lipid was increased with increased energy intake.

Added dietary cholesterol produced a significant ($P \leq 0.01$) increase in aorta scores for both the thoracic and abdominal segments as is seen in Table 4 and Figure 5. The energy x cholesterol interaction was significant ($P \leq 0.05$) for both

Figure 4. Effect of protein and energy restriction and dietary cholesterol on total liver lipid. Experiment 709

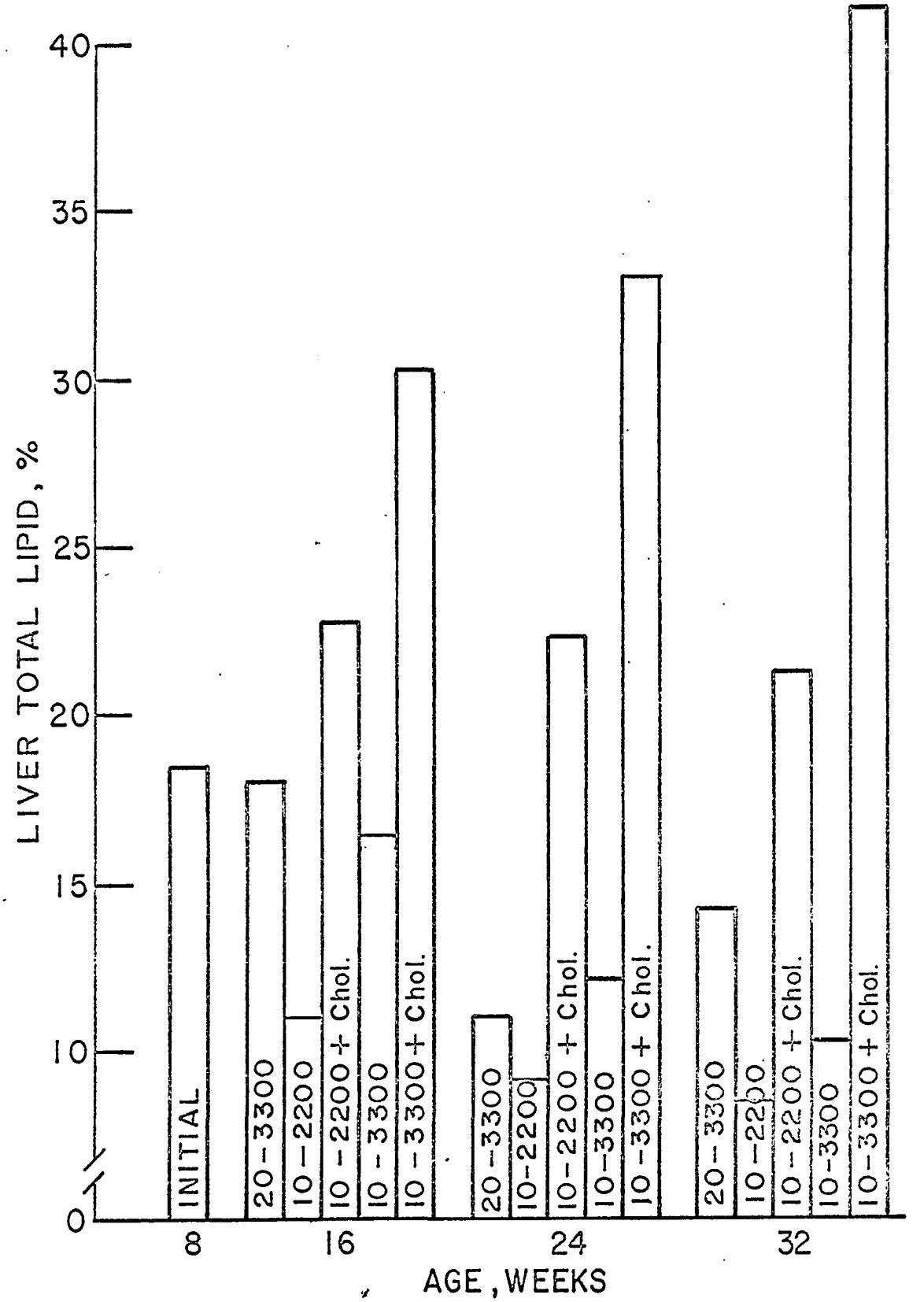
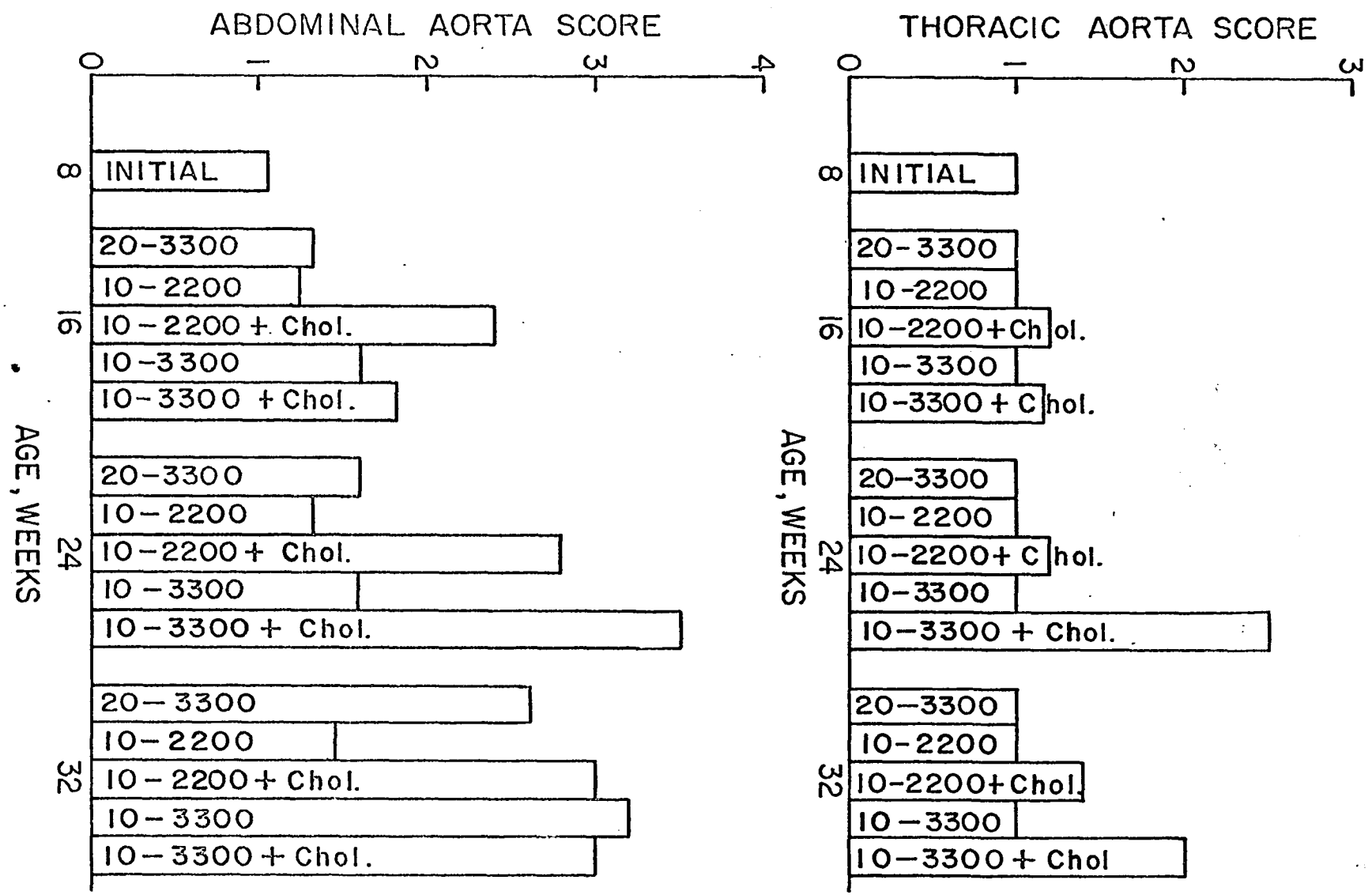

LIVER TOTAL LIPID, %
40
35
30
25
20
15
10
0
INITIAL
20-3300
10-2200
10-2200 + Chol.
10-3300
10-3300 + Chol.
20-3300
10-2200
10-2200 + Chol.
10-3300
10-3300 + Chol.
20-3300
10-2200
10-2200 + Chol.
10-3300
10-3300 + Chol.
8
16
24
32
AGE, WEEKS

Figure 5. Effect of protein and energy restriction and dietary cholesterol on aorta scores. Experiment 709


ABDOMINAL AORTA SCORE
0
1
2
3
4
AGE, WEEKS
8
INITIAL
16
20-3300
10-2200
10-2200 + Chol.
10-3300
10-3300 + Chol.
24
20-3300
10-2200
10-2200 + Chol.
10-3300
10-3300 + Chol.
32
20-3300
10-2200
10-2200 + Chol.
10-3300
10-3300 + Chol.
THORACIC AORTA SCORE
0
1
2
3
AGE, WEEKS
8
INITIAL
16
20-3300
10-2200
10-2200+Chol.
10-3300
10-3300 + Chol.
24
20-3300
10-2200
10-2200+ Chol.
10-3300
10-3300 + Chol.
32
20-3300
10-2200
10-2200+Chol.
10-3300
10-3300 + Chol

segments, however the increased atherosclerotic involvement with higher energy intake in the presence of dietary cholesterol is clearly evident for the thoracic segment. In the absence of dietary cholesterol there was a significant ($P \leq 0.05$) energy effect, an increase in aorta score, observed in those birds consuming the 10-3300 diet compared with those consuming the 10-2200 diets. This energy influence in the absence of dietary cholesterol was present in the abdominal segment only. The data of Figure 5 show the average aorta score of the eight-week-old birds, and it can be seen that aorta scores increase with age. This linear age relationship was found to be significant at $P \leq 0.05$ and $P \leq 0.01$ for the thoracic and abdominal segments respectively.

Aorta total lipid values, Table 5, were quite high for both segments of the aorta and were not significantly influenced by the dietary treatments. Aorta total lipid concentration was found to be only slightly higher for the abdominal segment compared with the thoracic segment after eight weeks of age. At eight weeks of age, however, the thoracic total lipid was only two-thirds that of the abdominal segment.

A significant ($P \leq 0.01$) linear increase with age in total protein extracted by the NaCl buffer was noted for both the abdominal and thoracic aorta segments. Abdominal buffer extract protein was somewhat higher at eight weeks of age than that of the thoracic segment. However, at 32 weeks of age

Table 5. Effect of dietary protein and energy restriction, with and without cholesterol, on the aorta total lipid concentration.[a] Experiment 709

Treatment	Age, weeks: Thoracic aorta segment 8	16	24	32	Trt. avg.[b]	Abdominal aorta segment 8	16	24	32	Trt. avg.[b]
20-3300		20.53	23.63	22.88	22.63		20.92	26.14	24.25	24.21
10-2200		19.59	22.11	23.72	21.97		23.06	26.70	23.85	24.78
10-2200 + Chol.[c]		17.34	23.44	21.69	20.82		21.70	27.93	27.46	25.70
10-3300		26.73	23.65	21.64	24.00		22.68	25.69	25.85	24.74
10-3300 + Chol.[c]		18.76	23.77	22.27	20.98		26.82	32.20	25.82	27.16
Age avg.[b]	24.08[d]	20.56	23.21	22.43	22.08	36.50[d]	23.38	27.11	25.46	25.32

[a] % of lipid-free dry weight.

[b] Treatment and age averages are averages of all observations in that category and not averages of age or treatment means.

[c] 1% cholesterol.

[d] These data were not used in statistical analysis.

thoracic buffer extract protein concentration was higher than the abdominal buffer extract protein concentration (Table 6). Increased protein consumption or energy consumption significantly ($P \leq 0.01$) increased the buffer extract protein concentration compared with the respective groups restricted in these nutrient intakes. Added dietary cholesterol did not influence buffer extract protein concentration.

Hydroxyproline concentration of the NaCl buffer extract was used as a measure of the soluble collagen removed by this fraction (Table 7). Soluble collagen concentration was approximately four times higher in the abdominal segment than in the thoracic segment. Both the thoracic and abdominal segments exhibited a significant ($P \leq 0.01$ and $P \leq 0.05$) linear decrease in soluble collagen with increasing age. This decrease was evident at 16 weeks and 24 weeks of age with no difference in soluble collagen concentration for either segment at 24 and 32 weeks of age. The dietary treatment did not significantly influence soluble collagen concentration although there was a trend for increased soluble collagen of the abdominal segment from birds restricted in protein.

Insoluble collagen was solublized by converting it to gelatin by autoclaving the sample in distilled water. Total protein in the autoclave extract was estimated and the hydroxyproline concentration was used again to estimate the collagen content. Tables 8 and 9 show the data on autoclave extract

Table 6. Effect of dietary protein and energy restriction, with and without cholesterol on the aorta buffer extract protein concentration.[a] Experiment 709

Treatment	Age, weeks: Thoracic aorta segment 8	16	24	32	Trt. avg.[b]	Abdominal aorta segment 8	16	24	32	Trt. avg.[b]
20-3300		97	112	118	111		94	113	113	109
10-2200		85	83	110	93		74	83	91	83
10-2200 + Chol.[c]		86	89	105	93		85	104	105	98
10-3300		85	101	118	101		96	98	110	101
10-3300 + Chol.[c]		94	114	114	105		86	122	108	101
Age avg.[b]	78[d]	87	97	113	100	84[d]	87	101	106	98

[a] mcg/mg lipid-free dry weight.

[b] Treatment and age averages are averages of all observations in that category and not averages of age or treatment means.

[c] 1% cholesterol.

[d] These data were not used in statistical analysis.

Table 7. Effect of dietary protein and energy restriction, with and without cholesterol, on the aorta buffer extract hydroxyproline.[a] Experiment 709

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	32	Trt. avg.[b]	8	16	24	32	Trt. avg.[b]
20-3300		0.557	0.428	0.408	0.450		2.260	2.290	2.186	2.243
10-2200		0.670	0.448	0.446	0.507		2.688	2.437	2.088	2.387
10-2200 + Chol.[c]		0.798	0.462	0.496	0.585		2.710	2.048	2.222	2.327
10-3300		0.716	0.500	0.552	0.589		2.528	2.046	2.446	2.340
10-3300 + Chol.[c]		0.647	0.610	0.517	0.586		2.408	2.635	2.285	2.388
Age avg.[b]	0.610[d]	0.687	0.472	0.485	0.545	2.240[d]	2.529	2.252	2.247	2.339

[a] mcg/mg lipid-free dry weight.

[b] Treatment and age averages are averages of all observations in that category and not averages of age or treatment means.

[c] 1% cholesterol.

[d] These data were not used in statistical analysis.

Table 8. Effect dietary protein and energy restriction, with and without cholesterol, on the aorta autoclave extract protein concentration.[a] Experiment 709

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	32	Trt. avg.[b]	8	16	24	32	Trt. avg.[b]
20-3300		145	231	160	184		282	384	271	317
10-2200		154	199	189	184		299	394	277	329
10-2200 + Chol.[c]		170	189	173	178		320	366	274	320
10-3300		181	203	131	172		314	382	271	322
10-3300 + Chol.[c]		153	253	168	174		298	397	288	308
Age avg.[b]	145[d]	162	209	164	178	258[d]	304	383	277	320

[a] mcg/mg lipid-free dry weight.

[b] Treatment and age averages are averages of all observations in that category and not averages of age or treatment means.

[c] 1% cholesterol.

[d] These data were not used in statistical analysis.

Table 9. Effect of dietary protein and energy restriction, with and without cholesterol, on the aorta autoclave extract hydroxyproline concentration.[a] Experiment 709

Treatment	Age, weeks: Thoracic aorta segment 8	16	24	32	Trt. avg.[b]	Abdominal aorta segment 8	16	24	32	Trt. avg.[b]
20-3300		17.54	19.66	15.77	17.68		40.89	47.43	46.15	45.43
10-2200		16.57	19.81	16.66	17.90		44.19	52.46	39.35	45.88
10-2200 + Chol.[c]		18.63	16.73	16.61	17.32		41.78	46.89	41.75	43.47
10-3300		18.04	19.59	12.86	16.83		46.54	47.91	40.47	44.97
10-3300 + Chol.[c]		17.88	24.60	15.87	17.98		43.25	51.63	41.91	43.87
Age avg.[b]	16.57[d]	17.80	19.48	15.57	17.53	35.92[d]	43.50	49.10	41.93	44.72

[a] mcg/mg lipid-free dry weight.

[b] Treatment and age averages are averages of all observations in that category and not averages of age or treatment means.

[c] 1% cholesterol.

[d] These data were not used in statistical analysis.

protein and hydroxyproline. Autoclave extract protein and hydroxyproline concentrations were highest in the abdominal aorta segment, there being 1.80 times as much protein and 2.55 times as much hydroxyproline in this fraction for the abdominal segment compared with the thoracic. These constituents appeared to increase with age from 8 weeks to 24 weeks, but a marked decrease was observed for both these constituents at 32 weeks of age. Since only the 16, 24, and 32 week data were analyzed statistically the linear regression components for these variables were not significant. Most of the sum of squares appeared as deviations from the linear regression. There was a trend for increased insoluble collagen on the low protein diets of Experiment 727 for both segments.

The elastin residue data, Table 10, show an inverse relationship with age compared with the insoluble collagen. Elastin residue decreased from 8 to 16 weeks of age then increased slightly in the birds sacrificed at 32 weeks of age. The inverse relationship with insoluble collagen is evident for the two segments; with 40% more elastin residue in the thoracic segment than the abdominal segment.

The dietary treatment main effects were not significant for the elastin residue data of either aorta segment. A significant ($P \leq 0.05$) energy x cholesterol interaction was observed for the thoracic aorta segment. Cholesterol appeared to increase the percent elastin residue in birds fed low energy diets but decreased it in birds fed high energy diets.

Table 10. Effect of dietary protein and energy restriction, with and without cholesterol, on the percent elastin residue of aorta. Experiment 709

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	32	Trt. avg.[a]	8	16	24	32	Trt. avg.[a]
20-3300		65.66	60.95	66.00	63.98		48.52	41.47	43.65	43.94
10-2200		68.94	65.07	61.89	65.04		47.42	47.44	45.73	46.87
10-2200 + Chol.[b]		67.61	66.96	67.17	67.25		51.11	48.03	44.63	47.92
10-3300		66.74	63.62	71.03	67.13		44.43	45.23	49.23	46.29
10-3300 + Chol.[b]		66.13	57.08	65.62	64.62		44.88	35.23	43.62	42.96
Age avg.[a]	69.96[c]	67.01	63.57	66.31	65.66	50.75[c]	47.05	44.73	45.30	45.68

[a]Treatment and age averages are averages of all observations in that category and not averages of age or treatment means.

[b]1% cholesterol.

[c]These data were not used in statistical analysis.

Ratios of insoluble to soluble collagen (I/S) were calculated from the hydroxyproline values of the autoclave extract and buffer extract for the several treatments and are shown in Table 11. The I/S ratio increased from 8 to 16 weeks of age and then decreased at 32 weeks. Since only data from 16 to 32 weeks of age could be analyzed statistically this linear trend was not significant and most of the sum of squares appeared as deviations from the linear regression. There also was observed a trend, which approached significance, for a decreased I/S collagen ratio when protein was restricted.

The ratio of elastin residue to total aorta collagen (calculated from the total hydroxyproline concentration of the aorta using the factor 7.46) decreased with age from 8 to 24 weeks for both segments (Table 12). At 32 weeks of age a marked increase in the ratio was observed however, resulting in non-significance for linear regression. Although the linear component of the regression of this ratio on age approaches significance at $P \leq 0.01$ with the thoracic aorta data, this is actually indicating a positive slope. This is the result of the high 32 week ratio and the fact that the eight week data was not included in the analysis. None of the dietary treatments had any effect on the elastin residue collagen ratio.

Correlation coefficients for all variables of the aorta analysis data aorta score, terminal systolic blood pressure and terminal serum cholesterol are given in Table 45 of the Appendix.

Table 11. Effect of dietary protein and energy restriction, with and without cholesterol, on the aorta insoluble/soluble collagen ratio. Experiment 709

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	32	Trt. avg.[a]	8	16	24	32	Trt. avg.[a]
20-3300		35.20	48.32	41.53	42.68		18.01	21.23	21.07	20.43
10-2200		26.87	44.91	45.40	40.26		16.75	21.83	19.07	19.55
10-2200 + Chol.[b]		23.44	37.01	33.77	31.41		15.46	22.95	18.83	19.08
10-3300		27.31	42.36	25.95	31.87		18.42	23.78	16.63	19.61
10-3300 + Chol.[b]		29.17	48.24	31.72	32.99		18.97	19.62	18.32	18.78
Age avg.[a]	25.14[c]	27.91	43.67	35.52	35.69	16.00[c]	17.58	22.18	18.77	19.48

[a]Treatment and age averages are averages of all observations in that category and not averages of age or treatment means.

[b]1% cholesterol.

[c]These data were not used in statistical analysis.

Table 12. Effect of dietary protein and energy restriction, with and without cholesterol, on the aorta elastin residue/collagen ratio. Experiment 709

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	32	Trt. avg.[a]	8	16	24	32	Trt. avg.[a]
20-3300		4.93	4.17	6.16	5.11		1.53	1.15	1.28	1.29
10-2200		5.44	4.61	5.06	4.98		1.37	1.18	1.55	1.35
10-2200 + Chol.[b]		4.72	5.35	5.92	5.33		1.56	1.38	1.41	1.45
10-3300		4.81	4.35	7.15	5.44		1.22	1.23	1.59	1.35
10-3300 + Chol.[b]		4.83	3.03	5.44	4.84		1.34	0.87	1.39	1.29
Age avg.[a]	5.88[c]	4.92	4.48	5.93	5.14	1.78[c]	1.39	1.20	1.44	1.35

[a]Treatment and age averages are averages of all observations in that category and not averages of age or treatment means.

[b]1% cholesterol.

[c]These data were not used in statistical analysis.

For the thoracic aorta segment data significant ($P \leq 0.05$) correlations were obtained for percent elastin residue and buffer extract protein, $r = -0.27$; and highly significant ($P \leq 0.01$) correlations were obtained for percent lipid and autoclave extract protein, $r = 0.32$; percent elastin residue and autoclave extract protein, $r = -0.75$; percent elastin residue and autoclave extract hydroxyproline, $r = -0.59$; buffer extract protein and buffer extract hydroxyproline, $r = -0.32$; and autoclave extract protein and autoclave extract hydroxyproline, $r = 0.60$.

With the abdominal aorta data a significant ($P \leq 0.05$) correlation was obtained for percent lipid and autoclave extract hydroxyproline, $r = 0.28$, while highly significant ($P \leq 0.01$) correlations were observed for the following factors: abdominal aorta score and buffer extract protein, $r = 0.40$; percent lipid with buffer extract protein, $r = 0.42$; and with autoclave extract protein, $r = 0.30$. Percent elastin residue was negatively correlated with buffer extract protein, $r = -0.30$; buffer extract hydroxyproline, $r = -0.38$; autoclave extract protein, $r = -0.40$ and autoclave extract hydroxyproline, $r = -0.54$; while positive correlations were found for buffer extract and autoclave extract hydroxyproline, $r = 0.41$; and autoclave extract protein and autoclave extract hydroxyproline, $r = 0.72$. The following correlations approached significance with the data of the abdominal aorta segment: aorta score and

percent lipid, r = 0.22; percent lipid and percent elastin residue r = -0.21 and buffer extract hydroxyproline and autoclave extract protein r = 0.21.

Significant correlations for corresponding variables between segments were observed for aorta scores r = 0.26, ($P \leq 0.05$), and the following which were significant at $P \leq 0.01$: percent elastin residue, r = 0.46; buffer extract protein r = 0.44; autoclave extract protein, r = 0.60; and autoclave extract hydroxyproline, r = 0.36. Numerous other apparently significant correlations were observed for other factors between segments.

A negative correlation between abdominal aorta score and terminal systolic blood pressure, r = -0.26, was the only association found with any of the data and blood pressure. None of the aorta analysis data was significantly correlated or approached significance with terminal serum cholesterol level.

Experiment 727

The purpose of Experiment 727 was to investigate the effect of reduced protein intake and the proportion of total calories from soybean oil in isocaloric diets, with and without added cholesterol, on certain physiological factors relating to atherosclerosis and the connective tissue components of the avain aorta. Weight gains and protein and energy consumption for the various experimental groups are shown in

Table 13.

Weight gains of birds fed the 10% protein diets were 28% less than those fed the 20% protein diets and birds fed the diets in which 40% of the calories were from soybean oil gained only 79% as much as those fed the diets with 10% of the calories from soybean oil. The addition of 1% cholesterol to the diets did not influence weight gains. Eighty-five percent of the weight gain occurred from 8 to 16 weeks of age. During the last six week period, weight loss was frequently a problem.

The experimental diets were available to the birds at all times and, in general, rather uniform energy consumption, when expressed on a body weight basis, occurred within each period. Expressed as M.E. Calories/kg body weight the birds ate about one-third more calories the first eight week period than during the rest of the experiment. Since energy consumption was quite constant within each period protein consumption by the birds fed the 10% protein diets averaged about half that of the birds fed the 20% protein diets, as shown in Table 13.

Dietary cholesterol significantly ($P \leq 0.01$) increased serum cholesterol and the severity of this hypercholesterolemia was significantly ($P \leq 0.01$) influenced by the dietary protein level. The data in Table 14 and Figure 6 indicate that severe hypercholesterolemia was obtained more rapidly and was more acute in the birds fed the low protein diets with added cholesterol, resulting in a highly significant ($P \leq 0.01$) protein x cholesterol interaction. Although not statistically

Table 13. Effect of dietary protein level, proportion of calories from fat, and dietary cholesterol on weight gain, protein and energy consumption of cockerels. Experiment 727

Treatment	Age, weeks 8-16	16-24	24-30	8-30
10-10				
Weight gain (gm)	1671	231	-91	1811
Prot./day/kg body wt. (gm)	5.57	3.54	3.37	4.23
M.E. Cal./day/kg body wt.	154	95	93	116
10-10 + Chol.[a]				
Weight gain (gm)	1774	344	97	2215
Prot./day/kg body wt. (gm)	5.50	3.52	3.56	4.32
M.E. Cal./day/kg body wt.	159	97	98	120
10-40				
Weight gain (gm)	1455	682	-318	1819
Prot./day/kg body wt. (gm)	5.32	3.58	2.76	3.99
M.E. Cal./day/kg body wt.	147	99	77	110
10-40 + Chol.[a]				
Weight gain (gm)	1617	336	-318	1635
Prot./day/kg body wt. (gm)	5.55	3.92	4.43	4.20
M.E. Cal./day/kg body wt.	154	109	123	129
20-10				
Weight gain (gm)	1841	395	310	2546
Prot./day/kg body wt. (gm)	10.80	7.02	7.96	8.65
M.E. Cal./day/kg body wt.	149	97	110	120
20-10 + Chol.[a]				
Weight gain (gm)	2161	457	136	2754
Prot./day/kg body wt. (gm)	11.02	7.24	7.30	8.63
M.E. Cal./day/kg body wt.	153	100	101	120
20-40				
Weight gain (gm)	1847	416	-260	2003
Prot./day/kg body wt. (gm)	10.62	6.78	7.48	8.37
M.E. Cal./day/kg body wt.	147	94	104	116
20-40 + Chol.[a]				
Weight gain (gm)	1912	173	-213	1872
Prot./day/kg body wt. (gm)	11.98	7.44	8.70	9.43
M.E. Cal./day/kg body wt.	152	103	121	126

[a]1% cholesterol.

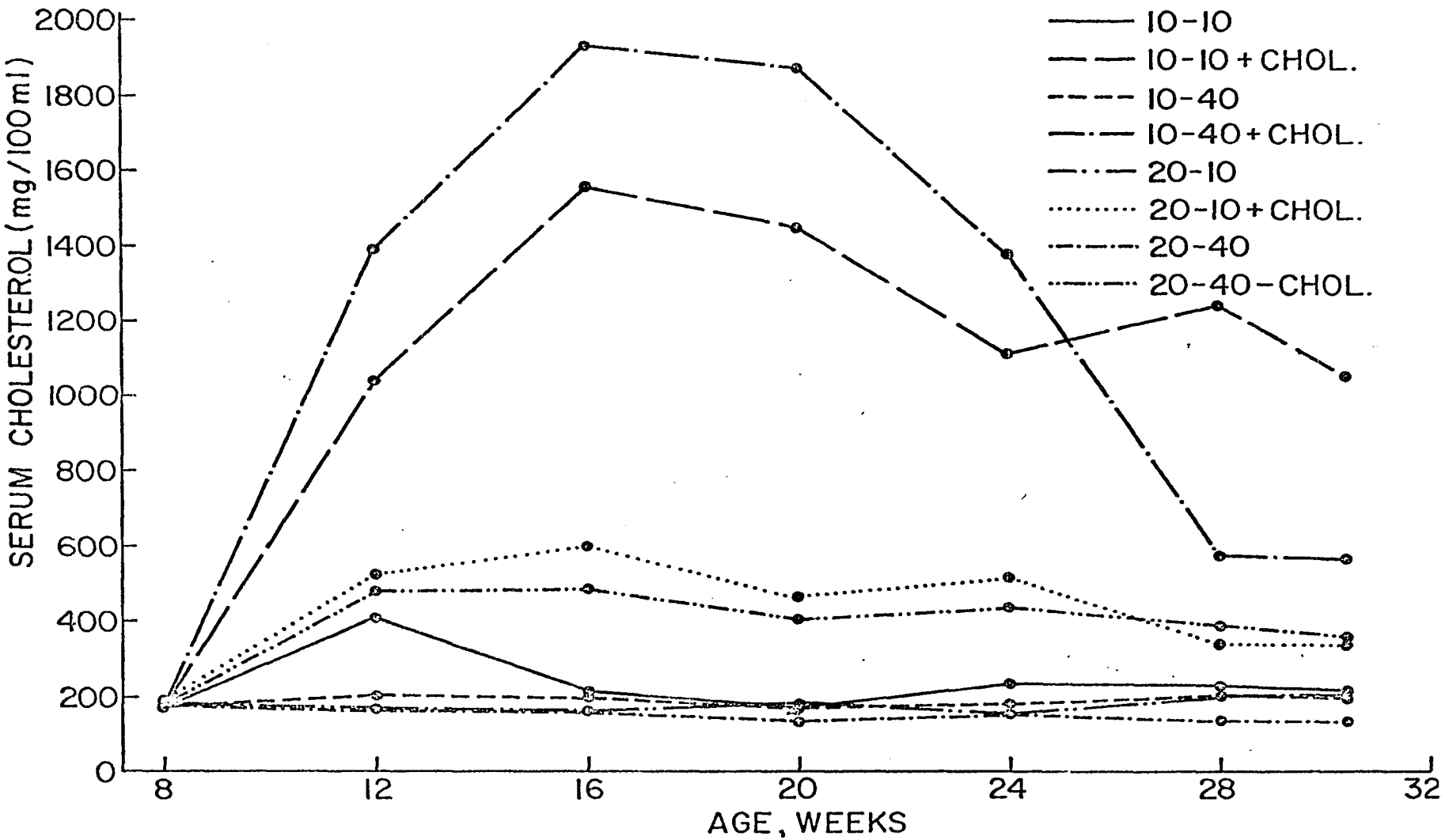
Table 14. Effect of dietary protein and proportion of calories from soybean oil with and without cholesterol on cockerels. Experiment 727

Treatment	Average data over 8-30 weeks			
	Thoracic aorta score	Abdominal aorta score	Serum cholesterol (mg/100 ml)	Liver[a] lipid (%)
10-10	1.00	1.50	245	19.73
10-10 + Chol.	1.70	2.70	1056	47.91
10-40	1.00	1.62	190	15.28
10-40 + Chol.	3.00	3.43	1268	54.01
20-10	1.00	1.60	157	13.26
20-10 + Chol.	1.00	1.62	390	30.90
20-40	1.00	1.57	171	14.21
20-40 + Chol.	1.14	2.14	440	47.57

[a]Dry weight basis.

significant there was a trend indicating an energy x cholesterol interaction. This was a tendency for hypercholesterolemia to be more severe, within each protein level, when a larger percentage of the calories came from soybean oil. When the diets contained no added cholesterol, serum cholesterol level of the birds fed the low protein diets appeared only slightly elevated compared to those fed the higher protein ration. As can also be seen in Figure 6 there appeared to be some adjustment to extreme hypercholesterolemia by birds fed diets containing

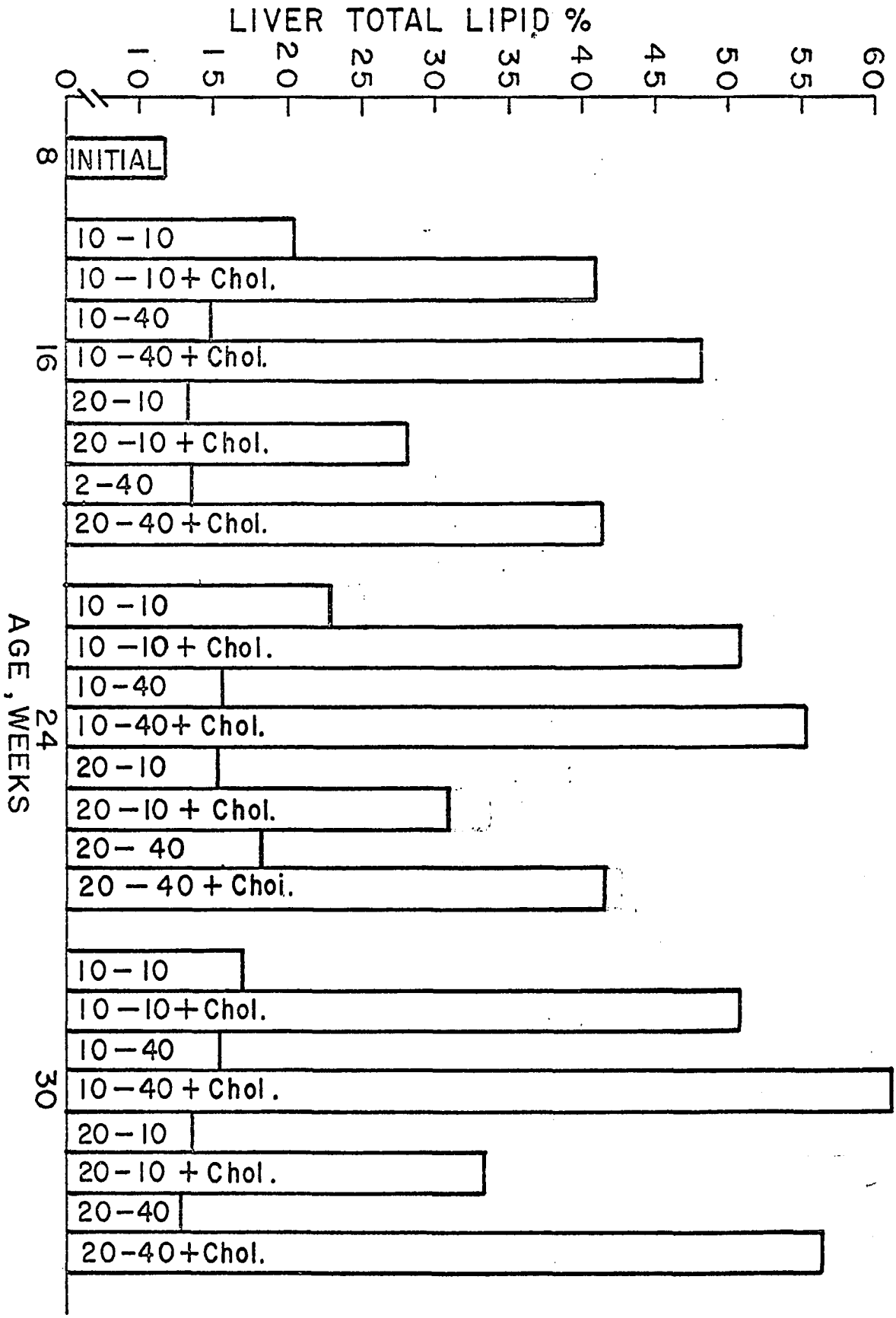
Figure 6. Effect of protein, percent of calories from soybean oil and dietary cholesterol on serum cholesterol concentration. Experiment 727


SERUM CHOLESTEROL (mg/100ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
8
12
16
20
24
28
32
AGE, WEEKS
10-10
10-10 + CHOL.
10-40
10-40 + CHOL.
20-10
20-10 + CHOL.
20-40
20-40-CHOL.

cholesterol, resulting in highly significant protein x age and cholesterol x age interactions.

Total liver lipid, Table 14 and Figure 7, was significantly ($P \leq 0.01$) increased by dietary cholesterol. Cholesterol-fed birds exhibited nearly three times as great a concentration. The magnitude of the cholesterol effect was, however, dependent on dietary protein and soybean oil content, and these interactions were highly significant ($P \leq 0.01$). Increasing dietary protein in the absence of dietary cholesterol resulted in a slight decrease (3.55%) in lipid concentration. In the presence of dietary cholesterol the birds fed the higher protein level had 12% less liver lipid than those fed low protein diets. The dietary soybean oil level, in the absence of dietary cholesterol, did not influence total liver lipid. However, with dietary cholesterol, liver lipid was 10% higher (40.5% vs. 50.75%) when the birds were fed high oil diets. A highly significant ($P \leq 0.01$) protein x oil interaction was observed because at the lower protein level, increasing the soybean oil level resulted in a 4% decrease in total liver lipid while with the higher protein diets increasing the dietary soybean oil level resulted in nearly a 10% increase in total liver lipid. A highly significant ($P \leq 0.01$) oil x age interaction also was observed since with the low soybean oil diets liver lipid did not change over the 16 to 30 week period, but with the higher soybean oil level an increase in liver lipid was observed with increasing age.

Figure 7. Effect of protein, percent of calories from soybean oil and dietary cholesterol on total liver lipid. Experiment 727


LIVER TOTAL LIPID %
0
10
15
20
25
30
35
40
45
50
55
60
AGE, WEEKS
8
INITIAL
16
10 – 10
10 – 10 + Chol.
10 – 40
10 – 40 + Chol.
20 – 10
20 – 10 + Chol.
2 – 40
20 – 40 + Chol.
24
10 – 10
10 – 10 + Chol.
10 – 40
10 – 40 + Chol.
20 – 10
20 – 10 + Chol.
20 – 40
20 – 40 + Choi.
30
10 – 10
10 – 10 + Chol.
10 – 40
10 – 40 + Chol.
20 – 10
20 – 10 + Chol.
20 – 40
20 – 40 + Chol.

Thoracic and abdominal aorta score data are shown as experimental averages and by periods in Table 14 in Figures 8 and 9. The thoracic scores indicated increased atherosclerotic involvement only when the diets contained cholesterol. This effect of dietary cholesterol was significantly ($P \leq 0.01$) influenced by the dietary protein and oil level and there was a significant ($P \leq 0.05$) protein x oil x cholesterol interaction. When the diets contained cholesterol, aorta scores were higher for birds fed the higher oil level or lower protein diets. The influence of dietary soybean oil was less when the diets contained 20% protein resulting in the significant three-way interaction. For the thoracic scores there was no difference due to age.

Again, for the abdominal aorta segment dietary cholesterol level was the prime influence on aorta score and this cholesterol effect was significantly ($P \leq 0.01$) influenced by dietary protein intake. Aorta scores were increased to a lesser extent when the birds consumed the higher protein diets. There was also a significant ($P \leq 0.05$) increase in abdominal aorta scores due to increased dietary soybean oil and a significant ($P \leq 0.01$) increase in aorta score with age.

Aorta total lipid was approximately 2.8% higher in the abdominal segment compared with the thoracic segment, Table 15. All three dietary factors had a significant ($P \leq 0.01$) influence on thoracic aorta total lipid. The increase in thoracic

Figure 8. Effect of protein, percent of the calories from soybean oil and dietary cholesterol on thoracic aorta scores. Experiment 727

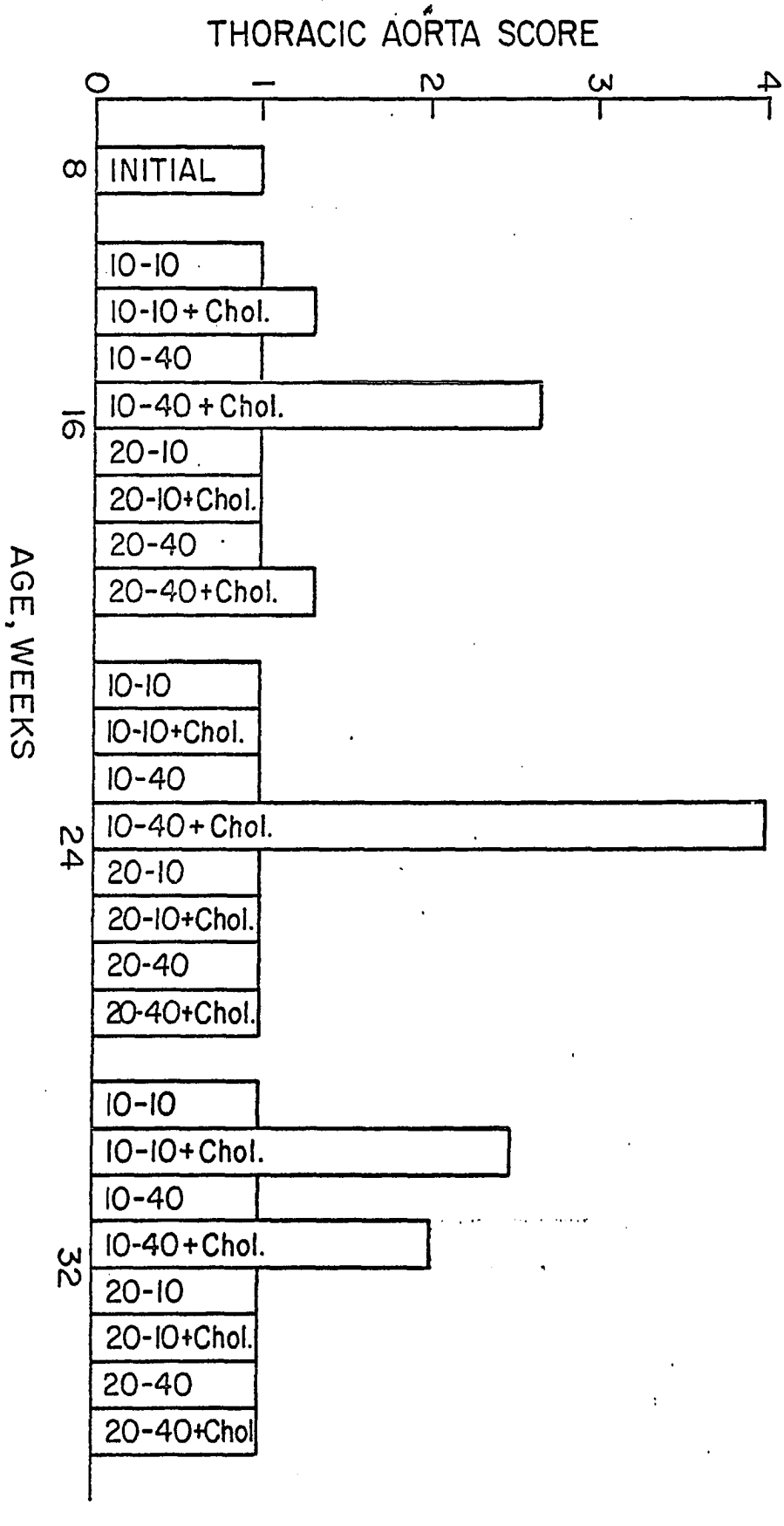

THORACIC AORTA SCORE
0
1
2
3
4
AGE, WEEKS
8
16
24
32
INITIAL
10-10
10-10+Chol.
10-40
10-40+Chol.
20-10
20-10+Chol.
20-40
20-40+Chol.
10-10
10-10+Chol.
10-40
10-40+Chol.
20-10
20-10+Chol.
20-40
20-40+Chol.
10-10
10-10+Chol.
10-40
10-40+Chol.
20-10
20-10+Chol.
20-40
20-40+Chol

Figure 9. Effect of protein, percent of calories from soybean oil and dietary cholesterol on abdominal aorta scores. Experiment 727

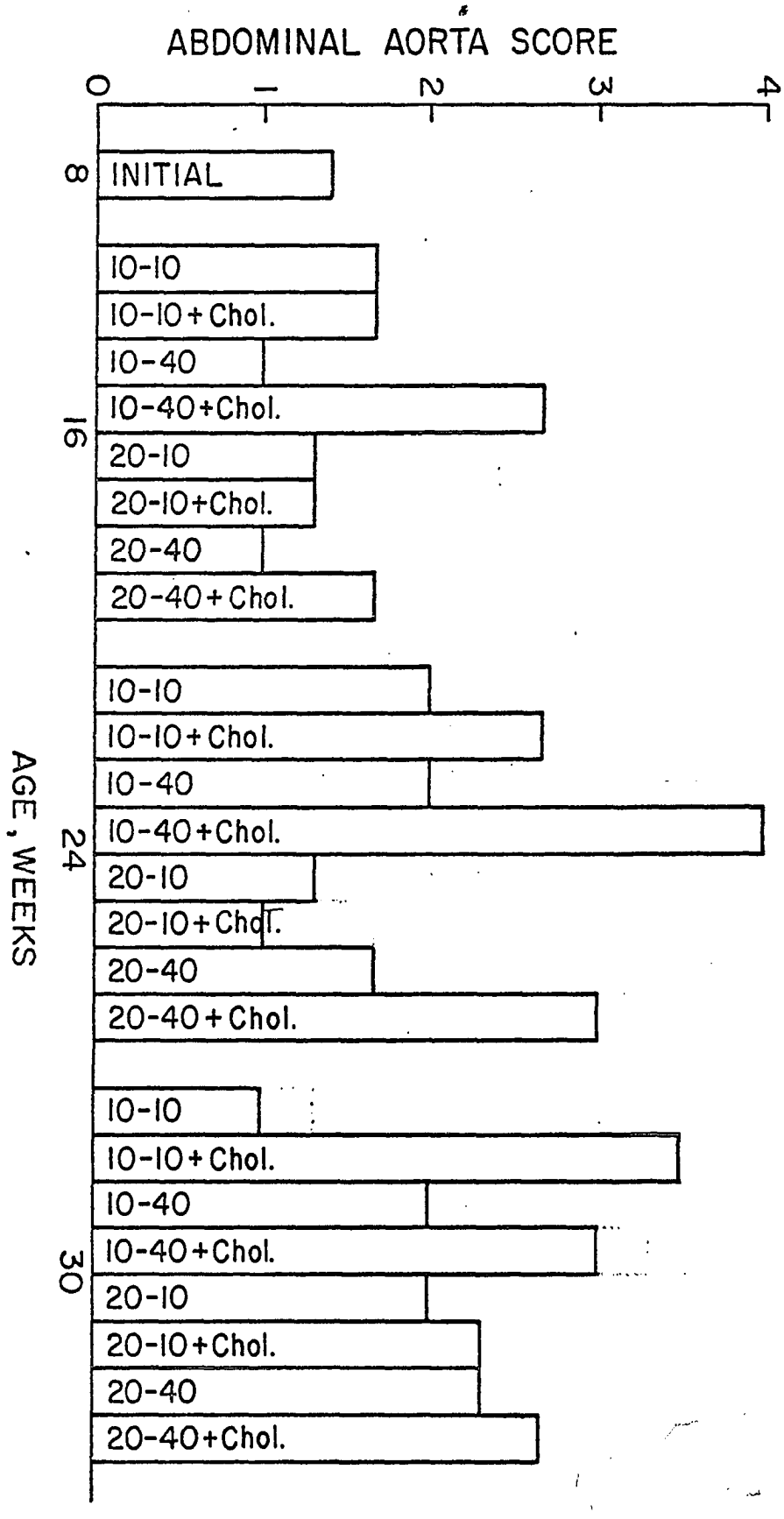

ABDOMINAL AORTA SCORE
0
1
2
3
4
AGE, WEEKS
8
16
24
30
INITIAL
10-10
10-10+Chol.
10-40
10-40+Chol.
20-10
20-10+Chol.
20-40
20-40+Chol.
10-10
10-10+Chol.
10-40
10-40+Chol.
20-10
20-10+Chol.
20-40
20-40+Chol.
10-10
10-10+Chol.
10-40
10-40+Chol.
20-10
20-10+Chol.
20-40
20-40+Chol.

Table 15. Effect of dietary protein, percent of the calories from soybean and dietary cholesterol[a] on the aorta total lipid concentration[b]. Experiment 727

Treatment	Age, weeks: Thoracic aorta segment 8	16	24	30	Trt. avg.[c]	Abdominal aorta segment 8	16	24	30	Trt. avg.[c]
10-10		4.96	3.45	3.88	4.34		10.17	5.03	6.56	8.11
10-10 + Chol.		6.01	4.38	6.22	5.74		11.23	6.02	11.36	9.71
10-40		4.19	4.66	4.12	4.35		7.76	6.60	5.28	6.70
10-40 + Chol.		8.96	8.43	4.36	7.40		16.80	12.41	9.94	13.57
20-10		4.04	3.83	3.43	3.73		5.69	5.00	3.94	4.78
20-10 + Chol.		4.60	4.05	4.88	4.56		10.43	3.86	3.88	6.82
20-40		4.99	4.54	3.94	4.68		8.04	4.58	3.82	5.92
20-40 + Chol.		5.00	5.08	3.46	4.36		10.02	5.95	3.38	6.59
Age avg.[c]	4.29[d]	5.32	4.77	4.48	4.88	7.91[d]	10.03	6.20	6.28	7.69
Main effect averages										
10% protein					5.49					9.50
20% protein					4.29					5.94
10% of the calories from soybean oil					4.63					7.30
40% of the calories from soybean oil					5.17					8.15
- cholesterol					4.23					6.18
+ cholesterol					5.51					9.15

[a] 1% cholesterol.

[b] % of lipid-free dry weight.

[c] Treatment and age averages are averages of all observations in that category and are not averages of age or treatment means.

[d] These data were not used in the statistical analysis.

aorta lipid due to dietary cholesterol was less for birds fed higher protein diets, resulting in a significant ($P \leq 0.01$) protein x cholesterol interaction. The main effect of soybean oil was to increase thoracic aorta lipid from 4.63% to 5.17%. In general there was a significant ($P \leq 0.05$) decrease in thoracic total lipid with age from 16 to 30 weeks with the eight week initial group exhibiting the lowest values. The significant ($P \leq 0.01$) oil x age interaction resulted because the birds fed the lower oil level exhibited a marked thoracic lipid reduction at 24 weeks of age compared with the decrease with age for birds fed higher oil diets.

Abdominal aorta total lipid was increased by feeding cholesterol and restricting protein, both main effects being highly significant ($P \leq 0.01$). This increase due to cholesterol was only about 30% as great when the diets contained 20% protein compared with 10% protein, resulting in a significant ($P \leq 0.05$) protein x cholesterol interaction. The observed 1% increase in abdominal aorta lipid in birds fed the higher oil level was not statistically significant. The initial group of birds sacrificed at eight weeks of age had about 7.9% total abdominal lipid compared with 10% for those birds sacrificed at 16 weeks of age. From 16 weeks there was a significant ($P \leq 0.01$) drop in abdominal aorta lipid in those birds sacrificed at 24 and 30 weeks of age with no difference between the 24 and 30 week groups.

The buffer extract protein data for the two aorta segments are shown in Table 16. For both segments there was a marked increase in buffer extract protein concentration from the birds sacrificed at eight weeks of age and those sacrificed at later periods. There was a trend for buffer extract protein to decrease in both segments from 16 to 30 weeks of age, but this was much more consistent and was significant in the thoracic segment only. A significant ($P \leq 0.05$) cholesterol x age interaction was found for the thoracic segment since without added cholesterol there was no change with age in buffer extract protein concentration while those birds fed dietary cholesterol showed a linear decrease with age. The abdominal segment data indicated a significant ($P \leq 0.05$) protein x cholesterol interaction because at the lower protein level buffer extract protein concentration increased with added dietary cholesterol while the opposite was true for birds fed the higher protein diets.

Buffer extract hydroxyproline concentration was nearly four times higher in the abdominal aorta segment compared with the thoracic aorta segment (Table 17). There was a significant ($P \leq 0.01$) linear decrease with age in buffer extract hydroxyproline for the thoracic aorta, this decrease being evident starting with those birds sacrificed at eight weeks of age. There was a similar trend between 16 and 30 weeks of age for the abdominal segment. However, the eight week initial group exhibited the lowest values of any age group. Thoracic aorta

Table 16. Effect of dietary protein, percent of the calories from soybean oil and dietary cholesterol[a] on aorta buffer extract protein concentration[b]. Experiment 727

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	30	Trt. avg.[c]	8	16	24	30	Trt. avg.[c]
10-10		85	88	84	85		93	73	75	84
10-10 + Chol.		104	89	97	97		100	93	96	96
10-40		86	86	92	88		89	80	89	86
10-40 + Chol.		102	102	70	93		95	101	95	96
20-10		88	90	87	88		99	97	80	90
20-10 + Chol.		95	92	81	89		81	100	82	86
20-40		94	87	88	90		87	97	88	91
20-40 + Chol.		95	93	84	90		89	80	86	86
Ave avg.[c]	58[d]	93	90	86	90	49[d]	92	91	78	90
Main effect averages										
10% protein					91					91
20% protein					89					88
10% of the calories from soybean oil					90					90
40% of the calories from soybean oil					90					90
- cholesterol					87					88
+ cholesterol					92					91

[a] 1% cholesterol.

[b] mcg/mg lipid-free dry weight.

[c] Treatment and age averages are averages of all observations in that category and are not averages of age or treatment means.

[d] These data were not used in the statistical analysis.

Table 17. Effect of dietary protein, percent of the calories from soybean oil and dietary cholesterol[a] on aorta buffer extract hydroxyproline concentration.[b] Experiment 727

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	30	Trt. avg.[c]	8	16	24	30	Trt. avg.[c]
10-10		0.205	0.263	0.120	0.187		0.724	0.715	0.725	0.723
10-10 + Chol.		0.230	0.223	0.155	0.199		0.874	0.837	0.620	0.762
10-40		0.258	0.196	0.150	0.209		0.697	0.818	0.737	0.754
10-40 + Chol.		0.277	0.171	0.188	0.223		0.791	0.604	0.646	0.696
20-10		0.174	0.179	0.157	0.168		0.809	0.758	0.718	0.757
20-10 + Chol.		0.265	0.183	0.154	0.205		0.729	0.668	0.625	0.672
20-40		0.175	0.153	0.136	0.157		0.767	0.785	0.766	0.779
20-40 + Chol.		0.195	0.189	0.146	0.174		0.803	0.790	0.665	0.740
Age avg.[c]	0.288[d]	0.223	0.194	0.152	0.190	0.590[d]	0.775	0.758	0.680	0.737
Main effect averages										
10% protein					0.205					0.737
20% protein					0.176					0.737
10% of the calories from soybean oil					0.189					0.733
40% of the calories from soybean oil					0.191					0.742
- cholesterol					0.180					0.754
+ cholesterol					0.200					0.720

[a] 1% cholesterol.

[b] mcg/mg lipid-free dry weight.

[c] Treatment and age averages are averages of all observations in that category and are not averages of age or treatment means.

[d] These data were not used in the statistical analysis.

buffer extract hydroxyproline was highly significantly ($P \leq 0.01$) increased and significantly ($P \leq 0.05$) increased in those birds fed the lower protein or cholesterol containing diets respectively, indicating more soluble aorta collagen for those groups. No significant treatment influences were observed for the abdominal aorta segment.

Table 18 contains the data collected on the protein concentration of the autoclave extract of the two aorta segments. Beginning with the eight-week-old birds there appears to be an increase in the protein concentration of this fraction with age. However, the concentration of protein measured from the 30-week-old group dropped below that found for the 24-week-old group. Since only the 16, 24 and 30 week data were analyzed statistically the significant ($P \leq 0.05$) age differences observed for both the thoracic and abdominal segments is quadratic in nature. There were no dietary treatment differences.

The hydroxyproline concentration in the aorta autoclave extract, which was used as a measure of insoluble collagen, was approximately twice as high in the abdominal segment as in the thoracic and was found to increase significantly ($P \leq 0.01$) with age in both segments (Table 19). This increase was continuous from 8 to 30 weeks of age with the largest change occurring between 8 and 16 weeks of age. There were no significant dietary treatment main effects, however there was a trend toward a higher thoracic autoclave extract hydroxyproline

Table 18. Effect of dietary protein, percent of the calories from soybean oil and dietary cholesterol[a] on aorta autoclave extract protein concentration[b]. Experiment 727

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	30	Trt. avg.[c]	8	16	24	30	Trt. avg.[c]
10-10		204	213	234	216		323	407	398	345
10-10 + Chol.		208	240	237	229		338	378	351	355
10-40		194	214	226	209		370	364	362	366
10-40 + Chol.		273	216	214	244		368	340	308	343
20-10		208	257	219	227		339	395	402	365
20-10 + Chol.		204	230	232	221		327	313	391	364
20-40		207	218	215	212		320	338	373	343
20-40 + Chol.		193	320	207	216		328	439	337	347
Age avg.[c]	159[d]	211	235	225	222	243[d]	340	375	355	355
Main effect averages										
10% protein					225					353
20% protein					220					356
10% of the calories from soybean oil					224					358
40% of the calories from soybean oil					220					350
- cholesterol					217					357
+ cholesterol					228					353

[a] 1% cholesterol.

[b] mcg/mg lipid-free dry weight.

[c] Treatment and age averages are averages of all observations in that category and are not averages of age or treatment means.

[d] These data were not used in the statistical analysis.

Table 19. Effect of dietary protein, percent of the calories from soybean and dietary cholesterol[a] on aorta autoclave extract hydroxyproline concentration[b]. Experiment 727

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	30	Trt. avg.[c]	8	16	24	30	Trt. avg.[c]
10-10		19.00	26.53	26.28	22.67		46.49	56.38	63.59	53.85
10-10 + Chol.		21.85	23.98	26.01	24.15		45.74	62.75	51.33	53.11
10-40		21.98	24.09	25.34	23.61		50.67	51.97	54.36	52.08
10-40 + Chol.		28.89	23.47	27.01	25.52		51.48	52.27	50.39	51.39
20-10		20.32	24.39	25.03	23.42		46.98	53.34	56.85	52.82
20-10 + Chol.		19.25	21.68	25.99	22.38		42.32	43.72	58.92	49.36
20-40		22.04	25.22	24.87	23.74		44.15	53.68	56.87	50.96
20-40 + Chol.		22.61	21.58	25.82	23.83		43.68	54.76	49.31	47.64
Age avg.[c]	14.76[d]	21.24	23.89	26.16	23.67	30.39[d]	46.54	54.06	54.90	51.49
Main effect averages										
10% protein					24.04					52.60
20% protein					23.32					50.41
10% of the calories from soybean oil					23.26					52.27
40% of the calories from soybean oil					24.16					50.57
- cholesterol					23.40					52.40
+ cholesterol					23.94					50.60

[a] 1% cholesterol.

[b] mcg/mg lipid-free dry weight.

[c] Treatment and age averages are averages of all observations in that category and are not averages of age or treatment means.

[d] These data were not used in the statistical analysis.

concentration for birds fed the lower protein diets. There was also a significant cholesterol x age interaction for the thoracic segment. This resulted because in the absence of cholesterol the largest hydroxyproline age increase occurred between 16 and 24 weeks while in those birds fed cholesterol the largest increase occurred between 24 and 30 weeks of age.

Elastin residue data, Table 20, show that there was more elastin residue in the thoracic aorta segment compared with the abdominal segment. Although there were no significant dietary treatment or age main effects for this segment, there was a trend for an increased thoracic elastin residue from the aortas of the birds fed the higher soybean oil diets. For this segment there was a significant ($P \leq 0.05$) oil x age interaction due to the linear decrease with age observed in birds fed the lower oil diets compared with the lack of any change in elastin residue percentage of those fed the higher oil diets. Elastin residue of the thoracic segment tended to decrease from 8 to 30 weeks. A similar but larger decrease with age in elastin residue percentage was observed for the abdominal segment and this change was highly significant ($P \leq 0.01$).

A ratio of insoluble to soluble collagen (Table 21) was calculated from autoclave extract and buffer extract hydroxyproline data to give an indication of the relationship of these two collagen forms. At eight weeks of age this ratio was similar for both segments and both segments exhibited significant ($P \leq 0.01$) increases in this ratio with age. The

Table 20. Effect of dietary protein, percent of the calories from soybean oil and dietary cholesterol[a] on percent elastin residue of aorta. Experiment 727

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	30	Trt. avg.[b]	8	16	24	30	Trt. avg.[b]
10-10		63.32	57.24	54.08	58.52		40.95	37.33	38.15	39.44
10-10 + Chol.		60.57	59.83	52.37	57.04		40.50	33.50	31.28	34.69
10-40		62.11	58.58	59.96	60.26		42.17	35.88	31.62	37.19
10-40 + Chol.		57.03	56.51	56.48	56.72		37.84	34.54	35.36	36.18
20-10		62.63	57.41	54.98	58.03		38.99	34.24	32.11	34.87
20-10 + Chol.		62.60	57.00	53.88	57.95		44.80	35.06	35.07	38.75
20-40		61.64	64.14	64.06	60.09		43.21	34.74	32.16	38.06
20-40 + Chol.		62.33	57.86	64.13	62.54		41.66	37.61	32.90	37.35
Age avg.[b]	70.23[c]	61.37	58.69	56.97	59.11	57.79[c]	41.42	34.97	33.28	36.84
Main effect averages										
10% protein					58.09					36.59
20% protein					60.10					37.08
10% of the calories from soybean oil					57.81					36.54
40% of the calories from soybean oil					60.64					37.19
- cholesterol					59.84					37.07
+ cholesterol					58.40					36.61

[a] 1% cholesterol.

[b] Treatment and age averages are averages of all observations in that category and are not averages of age or treatment means.

[c] These data were not used in the statistical analysis.

Table 21. Effect of dietary protein, percent of the calories from soybean oil and dietary cholesterol[a] on the aorta insoluble/soluble collagen ratio. Experiment 727

Treatment	Age, weeks: Thoracic aorta segment 8	16	24	30	Trt. avg.[b]	Abdominal aorta segment 8	16	24	30	Trt. avg.[b]
10-10		92.7	101.9	196.0	137.1		64.1	78.8	87.7	75.2
10-10 + Chol.		94.8	107.1	167.2	129.4		52.3	75.3	82.2	72.0
10-40		85.9	119.8	168.8	127.6		73.3	63.5	73.8	71.6
10-40 + Chol.		82.5	137.2	143.9	122.3		65.4	86.7	78.0	75.7
20-10		117.1	133.8	161.0	145.3		58.0	70.3	79.2	71.1
20-10 + Chol.		73.5	118.5	166.0	119.0		58.3	65.5	94.5	76.3
20-40		127.7	164.9	181.3	154.9		57.5	68.4	74.3	65.3
20-40 + Chol.		113.7	124.0	176.7	141.9		54.5	76.9	74.0	65.2
Age avg.[b]	48.4[c]	101.3	128.1	175.8	134.5	49.0[c]	61.7	72.9	81.4	71.6
Main effect averages										
10% protein					128.8					73.5
20% protein					140.1					69.9
10% of the calories from soybean oil					133.0					73.3
40% of the calories from soybean oil					136.4					69.5
- cholesterol					141.3					70.7
+ cholesterol					128.0					72.4

[a] 1% cholesterol.

[b] Treatment and age averages are averages of all observations in that category and are not averages of age or treatment means.

[c] These data were not used in the statistical analysis.

ratio increased much more for the thoracic segments, however, and at 30 weeks of age it was approximately twice that of the abdominal segment. Birds fed dietary cholesterol exhibited a significantly ($P \leq 0.05$) lower thoracic insoluble/soluble collagen ration, indicating a greater proportion of the total collagen in the soluble form. This was the only significant dietary treatment effect for either segment.

An elastin residue/collagen ratio was also calculated and the data of Table 22 show the marked differences between the two aorta segments for the ratio of these components. Between 16 and 30 weeks of age the thoracic segment had about 3.5 times as much elastin as collagen, while these components were approximately equal in the abdominal segment. For both segments at eight weeks of age there was a much greater proportion of elastin than at the later ages and both exhibited a highly significant ($P \leq 0.01$) decrease in this ratio with age. There was no significant dietary treatment alteration of this ratio, only a trend for a reduction in this ratio for the thoracic segment of the birds fed the lower protein diets.

The correlation coefficients calculated for the aorta scores, aorta analysis and terminal serum cholesterol level data of Experiment 727 are given in Table 66 of the Appendix.

Within the thoracic segment, percent lipid and aorta score were correlated with $r = 0.40$ ($P \leq 0.01$); buffer extract protein correlated with percent lipid, $r = 0.50$ and percent

Table 22. Effect of dietary protein, percent of the calories from soybean oil and dietary cholesterol[a] on the aorta elastin residue/collagen ration. Experiment 727

	Age, weeks									
	Thoracic aorta segment					Abdominal aorta segment				
Treatment	8	16	24	30	Trt. avg.[b]	8	16	24	30	Trt. avg.[b]
10-10		4.42	2.86	2.75	3.56		1.16	0.88	0.80	1.00
10-10 + Chol.		3.67	3.31	2.68	3.23		1.16	0.71	0.81	0.89
10-40		3.74	3.23	3.13	3.42		1.10	0.91	0.77	0.96
10-40 + Chol.		2.14	3.20	2.78	3.02		0.97	0.88	0.93	0.95
20-10		4.10	3.13	2.93	3.39		1.09	0.85	0.75	0.90
20-10 + Chol.		4.30	3.50	2.76	3.55		1.40	1.06	0.79	1.10
20-40		3.72	3.39	3.44	3.58		1.29	0.86	0.75	1.03
20-40 + Chol.		3.66	3.56	3.31	3.52		1.25	0.91	0.88	1.06
Age avg.[b]	7.00[c]	3.90	3.28	2.94	3.40	2.76[c]	1.21	0.87	0.82	0.98
Main effect averages										
10% protein					3.30					0.94
20% protein					3.50					1.01
10% of the calories from soybean oil					3.41					0.96
40% of the calories from soybean oil					3.39					1.00
- cholesterol					3.47					0.96
+ cholesterol					3.33					1.00

[a] 1% cholesterol.

[b] Treatment and age averages are averages of all observations in that category and are not averages of age or treatment means.

[c] These data were not used in the statistical analysis.

elastin residue, r = -0.33, both at $P \leq 0.01$; autoclave extract protein correlated with percent elastin residue r = -0.43 ($P \leq 0.01$) and buffer extract protein, r = 0.25 ($P \leq 0.05$); and autoclave extract hydroxyproline correlated with percent lipid, r = -0.40, percent elastin residue, r = -0.47, buffer extract protein, r = -0.34 and buffer extract hydroxyproline, r = -0.50, all significant at $P \leq 0.01$. Correlation coefficients approaching significance for variables of this segment were percent elastin residue and thoracic aorta score, r = -0.24; and buffer extract protein and aorta score r = -0.21, percent elastin residue, r = 0.24, and buffer extract protein, r = 0.24.

Highly significant ($P \leq 0.01$) correlation coefficients between the variables of the abdominal aorta segment were found for percent elastin residue and aorta score, r = -0.42; percent lipid, r = 0.34; and autoclave extract protein r = -0.54; and between autoclave extract hydroxyproline and percent lipid, r = -0.38, percent elastin residue r = -0.56, and autoclave extract protein r = 0.48. Buffer extract hydroxyproline and aorta scores were negatively correlated with r = -0.28 and the correlation between buffer extract protein and percent elastin residue, r = -0.21, approached significance.

Correlation coefficients between corresponding variables of the two segments were: aorta scores, r = 0.47; percent lipid, r = 0.54; percent elastin residue, r = 0.39; autoclave extract protein, r = 0.47; and autoclave extract hydroxyproline,

$r = 0.53$, all significant at $P \leq 0.01$. Similarly buffer extract protein concentrations correlated with a $r = 0.26$ ($P \leq 0.05$) and the correlation between buffer extract hydroxyproline concentrations for the two segments approached significance with a $r = 0.24$. Again quite a number of other apparently significant correlation coefficients were found between segments for certain other factors.

Terminal serum cholesterol level was significantly correlated ($P \leq 0.01$) with thoracic aorta score, $r = 0.42$; thoracic percent elastin residue, $r = 0.62$; thoracic buffer extract protein $r = 0.45$; thoracic autoclave extract hydroxyproline, $r = 0.32$; and abdominal percent lipid, $r = 0.41$. Correlations approaching significance were observed between terminal serum cholesterol and thoracic autoclave extract protein, $r = -0.23$, abdominal buffer extract protein, $r = 0.22$, and abdominal autoclave extract hydroxyproline, $r = -0.21$.

GENERAL DISCUSSION

With these two experiments attempts were made to study physiological influences of varying protein and energy intakes and energy source. The energy restriction regime used in Experiment 709 apparently was quite severe and retarded growth much more than the level of protein restriction imposed. Protein restriction was obtained to a varied extent on the 10% protein diets compared with the 20% basal diets, however, little can be concluded from this experiment from the energy restriction. Energy intake was restricted on a daily basis but not on a body weight basis, as was desired. Confounding this daily energy restriction is the fact that birds fed the 10-3300 diets had a protein intake of only 2/3 that of the birds fed the 10-2200 diets on a body weight basis although they had similar total daily protein intakes. Consequently it cannot be stated that true energy influences exist.

Protein restriction in Experiment 727 was more uniform because the diets were isocaloric and the birds were allowed to eat ad libitum. Weight gains were reduced by protein restriction and by the higher percentage of calories from soybean oil.

As would be expected from much previous work, dietary cholesterol had a profound influence on serum cholesterol. These results support the work of Rose (1967) in that protein restriction increased and energy restriction decreased serum cholesterol when the diets contained added cholesterol. In Experiment 709 the birds fed the 10-3300 + Chol. diets had a

much higher serum cholesterol level than those fed the 10-2200 + Chol diets. This could be due to the restricted protein or increased energy consumption of the 10-3300 + Chol fed group as discussed previously. The protein effect is quite clear in Experiment 727 and the data of Figure 6 support the observations of Nichols (1963) that high levels of soybean oil tend to increase serum cholesterol.

The blood pressure differences observed in Experiment 709 are probably associated with the corresponding body weight differences. Both Nichols (1963) and Rose (1967) concluded that blood pressure difference was more closely associated with body weight than with dietary treatments.

Total liver lipid was significantly increased in each age group for both experiments when the diets contained cholesterol. The analytical data as well as the appearance of the livers from birds fed cholesterol indicated that 1% cholesterol severely overloaded the birds system resulting in abnormally high lipid concentrations. Increasing dietary protein in Experiment 727 reduced liver lipid; to the greatest extent in the presence of cholesterol. This effect was not observed in Experiment 709 when the 20-3300 and 10-2200 and 10-3300 diets were compared. The increased liver lipid noted in response to the higher daily energy intake in this experiment may, however, have been partially due to the lower protein intake of those birds fed the higher energy diets. The higher liver lipid concentration of birds in Experiment 727 attributed to soybean oil level was

observed only in the presence of cholesterol and this may have been due to oil increasing cholesterol absorption.

Aorta scores in both experiments were based on visual examination of the unstained segments. This is a subjective measure at best and without staining, as is usually done, the grader will probably see only the quite severe yellow lipid deposits and fibrous plaques. In spite of this handicap; necessary to preserve the tissues for later analysis; marked treatment differences in aorta scores were observed. In both experiments abdominal segments were more severely afflicted than thoracic segments and, as would be expected, cholesterol was highly effective in inducing atherosclerosis. The significant energy x cholesterol interaction and energy effect observed for the thoracic and abdominal segment respectively in Experiment 709 is in agreement with Rose (1967). However, as discussed previously, this apparent energy effect could not be separated from a possible influence of protein intake.

The increases in cholesterol-induced thoracic aorta atherosclerosis due to a high soybean oil intake or restricted protein intake observed in Experiment 727 are in agreement with the results of Nichols (1963) and Rose (1967). All dietary treatments significantly influenced abdominal aorta scores and the significantly increased severity of the cholesterol induced lesion due to restricted protein supports the trends observed by Rose (1967).

There were significant age increases for aorta scores with the exception of the thoracic scores in Experiment 727. This response was observed mainly for those diets containing cholesterol. However, there were age trends in the absence of cholesterol for the abdominal segment.

Aorta total lipid values were not at all comparable between the two experiments. In Experiment 709 aorta total lipid was four to five times higher than in Experiment 727 and no reason can be given for this very marked difference. Because of the extremely high concentrations no significant treatment or age differences were observed in Experiment 709. More reasonable values prevailed in Experiment 727 and segment differences and treatment and age differences were observed. As has been reported by other workers, dietary cholesterol significantly increased aorta lipid. For each segment the higher protein level gave some protection against this lipid increase. Increased aorta lipid was observed in the birds fed the higher soybean oil diets; this effect was significant for the thoracic segment only although the difference was larger for the abdominal segment. The general decrease in aorta lipid between 16 and 32 weeks of age could be an adjustment to the dietary treatment or possibly due to removal of the more severely afflicted birds by sacrifice and mortality.

Buffer extract protein, a measure of the serous protein and soluble collagen, was in general quite comparable between the two segments and two experiments. Protein concentration of

this fraction was somewhat higher for the eight week group in Experiment 709 while this concentration was only about 10% higher at later ages. Significant increases with age were seen for both segments in Experiment 709 while the trend was just the opposite in Experiment 727. The only significant treatment differences were observed in Experiment 709 where the birds receiving the basal diet or higher energy diets had a higher concentration of protein in this buffer extract. This might be expected since this concentration appears related to rate of growth for the various groups and newly synthesized protein would probably be more soluble than "older" protein. This is in agreement with the report by Gross (1958) that static weight maintenance reduces the nonscleroprotein extracted from guinea pig dermis.

Marked differences were once again observed between the two experiments in the hydroxyproline concentration of the NaCl buffer extract. Although total protein values were comparable, soluble collagen concentration was approximately three times higher in this fraction in Experiment 709. The segment differences in soluble collagen were of the same magnitude in both experiments however, there being about four times as much in the abdominal segment. Soluble collagen decreased with age between 16 and 30 weeks of age in both segments. Similar results were reported by McGavack and Kao (1960) for rat aorta tissue. The soluble collagen was less at eight weeks than at 16 weeks except for the thoracic segment of Experiment 727.

This should have been the period of most rapid growth and thus the highest values. Only in the case of the thoracic segment of the birds in Experiment 727 were significant dietary effects observed. Soluble collagen was increased in those birds fed the lower protein or cholesterol containing diets. A similar trend was apparent in Experiment 709 for the abdominal segment of birds on the restricted protein intake. One might theorize that this suggests protein restriction or cholesterol induced atherosclerosis may inhibit collagen maturation. According to the report of Grant (1958), however, if protein restriction reduced growth as it did do in these experiments one would expect a reduced collagen synthesis and thus reduced soluble collagen.

The total protein concentration in the autoclave extract was about two and three times that of the buffer extract for each experiment. The total protein of the buffer and autoclave extracts agrees fairly well with the data of Gan et al. (1967) for total nonelastin protein in the abdominal and thoracic aorta. These workers also reported a significant linear increase in total nonelastin protein between three and thirty six months. This later observation of age changes was noted in these experiments from 8 weeks to 24 weeks. However, at 32 and 30 weeks of age rather marked decreases were found in autoclave extract protein resulting in a total aorta protein decrease. The reason for this discrepancy may be that at this age a number of the birds were losing weight and this may have resulted

in abnormal values. No dietary treatment differences were observed for either segment in regard to autoclave extract protein.

A linear and quadratic increase in autoclave extract hydroxyproline was observed in Experiments 709 and 727 respectively between 8 and 24 weeks of age. In Experiment 709 there was a rather marked decrease at 32 weeks of age while there was a further slight increase at 30 weeks of age in Experiment 727. This drop in Experiment 709 again might be explained as due to weight loss of some birds during the last period. However, a similar problem occurred in Experiment 727 at this time but no reduction was observed. Another possibility is the autoclaving period was insufficient to solubilize all the remaining collagen in the aortas from this collection of Experiment 709. Insoluble collagen was reported to increase with age in the aorta of male rats up to eight months of age by McGavack and Koa (1960) and this study would agree in general with that report.

Gan et al. (1967) reported increases with age in the total collagen of chicken aorta. Insoluble collagen would be expected to increase and soluble collagen decrease because with age crosslinking of the collagen fibrils is thought to occur, reducing its extractability. Abdominal aorta total collagen values of these experiments are in general agreement with those of Gan et al. (1967), however total thoracic collagen is only about two-thirds of that reported. No significant treatment effects were observed.

Gan et al. (1967) reported elastin in terms of hydroxyproline (some workers assume elastin contains approximately 1.5% hydroxyproline, others consider it an indication of collagen contamination) and found a marked segment difference and decrease with age for both segments. Similar trends, significant in the case of the abdominal segment of Experiment 727, were observed in these experiments for the elastin residue data from eight weeks to 32 weeks of age. In experiment 709 this trend was again evident, however there was an increase in elastin residue at 32 weeks of age. This corresponded to the decrease in insoluble collagen observed. This group of aortas may not have been autoclaved for a sufficient length of time to solublize all the collagen and remove it. Consequently it appeared in the elastin residue. Hydroxyproline concentration was measured in several elastin residue samples to check for collagen contamination and on these samples was found to be less than 2%. Apparently the autoclaving was not sufficient for this group. The much higher elastin content of the thoracic aorta corresponds to very marked tissue difference observed visually for these segments. No significant treatment main effects were observed for either segment in these experiments in regard to elastin residue.

The insoluble/soluble collagen ratio increased with age for each segment in both experiments indicating a change to a more insoluble collagen form. This age change was not observed for the 32 week group of Experiment 709 probably because of the

aberrant values as previously discussed. Also the ratio was three to four times higher in Experiment 727 simply because much less soluble collagen was extracted from all groups. The insoluble to soluble collagen ratio was twice as high in the thoracic aorta segment compared with the abdominal for both experiments. Although total collagen was about four times greater in the abdominal segment, a greater proportion was in the soluble form. A trend for a decreased insoluble/soluble collagen ratio was observed for the thoracic segment with the restricted protein diets of Experiment 709 and the cholesterol fed groups of Experiment 727 had a significantly lower ratio for this segment. This may indicate a failure of the collagen to "mature" as rapidly when protein is restricted or in cholesterol induced atherosclerosis of the thoracic aorta.

The rather marked segment differences in the relative proportion of elastin and collagen observed are in agreement with previous observations. Harkness (1957) noted in dogs that the proportion of elastin is greater in the thoracic segment and Grant (1967) reported similarly in several other mammalian species that elastin decreased and collagen increased as the aorta was descended. Although the elastin residue/collagen ratio was somewhat higher in Experiment 709 compared with Experiment 727, in each experiment similar segment differences were observed. This ratio indicated that there was 3.5 times as much elastin residue as collagen in the thoracic segment compared with the abdominal segment. Gan et al. (1967)

expressed this segment difference as a collagen/elastin ratio calculating it from the hydroxyproline concentration of each of these components. This leads to quite different values compared with this experiment, first because the ratio is inverted and secondly, using hydroxyproline, values will not give a weight to weight ratio as is expressed for these experiments because hydroxyproline concentration is about ten times greater in collagen than in elastin. Recalculating from the data of Gan et al. (1967), an elastin/collagen ratio very comparable to the thoracic ratio of Experiment 727 and abdominal ratio of Experiment 709 was obtained. The segment differences for their ratios were slightly lower than in this experiment, 2.0-2.5 times higher for the thoracic than the abdominal but comparable to 3.0-3.5 reported for this experiment. Possible breed differences and the different techniques used may explain part of this difference. Only in Experiment 727 was a dietary treatment influence observed, a trend for a reduced ratio in those fed on low protein diets.

The within treatment correlation coefficient indicated several rather consistent associations in these two experiments, however there is little from the literature for comparison.

Aorta score was significantly positively correlated with percent lipid only for the thoracic segment in Experiment 727, however this correlation approached significance in the abdominal segment of Experiment 709. Significance in Experiment 727 was probably due to the high soybean oil diets which result in

increased deposition of lipid especially in the thoracic aorta segment. Both percent elastin residue and buffer extract protein were significantly negatively correlated or approached significance in correlation to aorta score for both segments in Experiment 727. Buffer extract hydroxyproline however is only a very small proportion of total hydroxyproline or collagen of the aorta. Weiss and Fisher (1959) reported that aortic hydroxyproline was very important in evaluating abdominal aorta atherosclerosis, while aortic cholesterol concentration was more closely associated with thoracic aorta atherosclerosis.

Percent aorta lipid was positively correlated in both segments with autoclave extract total protein in Experiment 709. In Experiment 727 both aorta segments showed significant negative correlation between percent lipid and autoclave extract hydroxyproline. The percent lipid and autoclave extract hydroxyproline were also significantly correlated in Experiment 709, however in this experiment the correlation was positive.

In both experiments percent elastin residue was negatively correlated in both segments with buffer extract protein, autoclave extract protein and autoclave extract hydroxyproline. This would be expected since the analysis involved partitioning the lipid free aorta tissue into these three fractions and their relative proportion should vary inversely.

Thoracic aorta buffer extract protein appeared to be correlated with the buffer extract hydroxyproline concentration of the same segment in both experiments but was inconsistent.

This was a negative correlation in Experiment 709 and a positive correlation in Experiment 727. The rather marked difference in buffer extract hydroxyproline concentration, 2.5 times as much in Experiment 709, may have influenced this result. In Experiment 727 thoracic segment buffer extract protein was also positively correlated with autoclave extract protein, and both buffer extract protein and hydroxyproline were negatively correlated with autoclave extract hydroxyproline. This would seem to indicate total protein in these two fractions varied together but hydroxyproline or collagen was partitioned into one or the other fractions. In the abdominal segment in Experiment 709, however, buffer extract hydroxyproline was positively correlated with autoclave extract hydroxyproline.

Autoclave extract protein was significantly correlated with autoclave extract hydroxyproline in the thoracic segment of Experiment 709 and in the abdominal segment of both experiments. This would be expected if a large part of the protein in the autoclave extract is collagen as undoubtedly is the case.

There was a very high degree of correlation between corresponding observations and aorta constituent concentrations between the two segments. Only in the case of percent lipid and buffer extract protein in Experiment 709 were these constituents not significantly correlated or nearly so between segments.

Correlations between aorta scores or aorta analysis components with either terminal serum cholesterol or terminal blood pressure were nearly entirely absent in Experiment 709. The only significant correlation was an apparent negative relationship between blood pressure and abdominal aorta score. This complete lack of correlation and especially the negative relationship was not expected, considering that Weiss and Fisher (1959) indicated blood pressure was associated with abdominal atherosclerosis. In Experiment 727 terminal serum cholesterol, however, was positively correlated with thoracic aorta score and in both segments with percent lipid and buffer extract protein.

In this latter experiment serum cholesterol concentration was also negatively correlated with autoclave extract protein of the thoracic segment and autoclave extract hydroxyproline of both segments. Since the relative condition or health of the birds in Experiment 727 was better than those severely restricted in Experiment 709, this may have resulted in more consistent relationships. In both experiments the high aorta scores, especially in the thoracic segment, were associated almost exclusively with those treatments resulting in high serum cholesterol levels so a high correlation between the two measurements would have been expected. The positive relationship between buffer extract protein and serum cholesterol level in Experiment 727 might suggest an effect on the protein components

of the aorta due to this stress, however, the inconsistent relationship between percent lipid and the protein and hydroxyproline in the various fractions did not support this.

SUMMARY

Two experiments were conducted in which varying protein and energy intakes and energy source, with and without dietary cholesterol, were studied for their influence on physiological factors associated with atherosclerosis and aging. Systolic blood pressure, serum cholesterol concentration and aorta atherosclerosis were determined and aorta tissue samples were subjected to a fractionation scheme in which total lipid, neutral-salt soluble and insoluble collagen and protein, and elastin residue were determined. Broiler type chickens between eight and thirty two weeks of age were used.

Energy restriction reduced growth quite severely compared with protein restriction in Experiment 709. In Experiment 727, when isocaloric diets were fed ad libitum, weight gains were reduced by protein restriction and when a higher percentage of the calories came from soybean oil.

Dietary cholesterol had a profound influence on both serum cholesterol and total liver lipid. In the presence of dietary cholesterol, protein restriction increased while energy restriction decreased serum cholesterol. Protein restriction, increased energy intake or a higher percentage of calories from soybean oil all resulted in a higher total liver lipid concentration when the diets contained added cholesterol.

Blood pressure differences observed in Experiment 709 were increased in relation to daily energy intake, however, systolic

blood pressure was probably associated with body weight differences rather than with dietary treatments.

Cholesterol-induced atherosclerosis was more severe in the abdominal than the thoracic segment. Energy restriction reduced cholesterol-induced atherosclerosis for the thoracic segment, while protein restriction or an increased percentage of calories from soybean oil increased this for both segments. Total aorta lipid was increased by dietary cholesterol and in those birds fed high soybean oil diets. Increased dietary protein gave some protection against this aorta lipid infiltration.

Marked aorta segment differences were observed for total protein, collagen and elastin. The thoracic aorta segment had an elastin residue/collagen ratio of 3.5 to 4 times as high as the abdominal segment. This difference was reflected in the relative amounts of total protein and total hydroxyproline in the buffer and autoclave extracts. Buffer extract protein was comparable between segments, however, autoclave extract protein was 1.60 to 1.75 times higher in the abdominal segment compared with the thoracic segment. The major portion of the collagen was insoluble in the neutral-salt buffer and decreases in the soluble collagen fraction were noted with age as evidenced by the increasing insoluble/soluble collagen ratio.

Few significant differences in the various fractions, other than age changes, were observed. Buffer extract protein in Experiment 709 was significantly increased in both segments due to increased protein or energy intake which resulted in

better growth. Soluble collagen was significantly increased in Experiment 727 for those birds fed the lower protein or cholesterol containing diets and a similar trend was apparent in Experiment 709 for birds fed the restricted protein diets.

Correlation coefficients indicated rather consistent relationship between several of the aorta analysis fractions within a segment and significant positive correlation between segments for the aorta analysis data. Only in Experiment 727 were significant positive correlations obtained between terminal serum cholesterol and thoracic aorta score, and in both segments for terminal serum cholesterol, and percent aorta lipid and buffer extract protein.

BIBLIOGRAPHY

Ayer, J. P.
1964 Elastic tissue. In Hall, D. A., ed. International review of connective tissue research. Vol. 2. Pp. 33-100. New York, New York, Academic Press.

Balo, J.
1963 Connective tissue changes in atherosclerosis. In Hall, D. A., ed. International review of connective tissue research. Vol. 1. Pp. 241-306. New York, New York, Academic Press, Inc.

Banerjee, S., P. N. Rao and S. K. Ghosh
1965 Biochemical and histochemical changes in aorta of chicks fed vegetable oils and cholesterol. Society for Experimental Biology and Medicine Proceedings 119: 1081-1086.

Banting, C. N. and H. Banting
1953 Acid mucopolysaccharides of the aorta. Archives of Pathology 55: 257-264.

Bertelsen, S.
1961a Alterations in human aorta and pulmonary artery with age. Acta Pathologica et Microbiologica Scandinavica 51: 206-228.

Bertelsen, S.
1961b Histochemical investigations on plaques in human atherosclerotic aortae. Acta Pathologica et Microbiologica Scandinavica 51: 229-240.

Bertelsen, S.
1962 Hexosamine, hydroxyproline and calcium levels in the intima of the human aorta as related to age and atherosclerotic changes. Journal of Gerontology 17: 24-26.

Bertelsen, S.
1963 The role of ground substance, collagen, and elastic fibers in the genesis of atherosclerosis. In Sandler, M. and G. H. Bourne, eds. Atherosclerosis and its origin. Pp. 119-165. New York, New York, Academic Press.

Bertelsen, S. and C. E. Jensen
1960 Histochemical studies on human aortic tissue. Acta Pathologica et Microbiologica Scandinavia 48: 305-315.

Bloom, William and Don W. Fawcett
1962 A textbook of histology. 8th ed. Philadelphia, Pennsylvania, W. B. Saunders Co.

Boucek, R. J., N. L. Noble and J. F. Woessner, Jr.
1959 Properties of fibroblasts. In Page, I. H., ed. Connective tissue, thrombosis and atherosclerosis. Pp. 193-211. New York, New York, Academic Press.

Branwood, A. W.
1963 The fibroblast. In Hall, D. A., ed. International review of connective tissue research. Vol. 1. Pp. 1-28. New York, New York, Academic Press.

Brimacombe, J. S. and J. M. Webber, eds.
1964 Mucopolysaccharides, chemical structure, distribution and isolation. Amsterdam, The Netherlands, Elsevier Publishing Co.

Buddecke, E.
1962 Chemical changes in the ground substance of the vessel wall in atherosclerosis. Journal of Atherosclerosis Research 2: 32-46.

Burton, A. C.
1954 Relation of structure to function of the tissues of the wall of blood vessels. Physiological review 34: 619.

Cembrano, J., M. Lillo, J. Val and J. Mardones
1960 Influence of sex difference and hormones on elastin and collagen in the aorta of chickens. Circulation Research 8: 527-529.

Cohen, A. M. and S. Shoshan
1968 Effect of glucose, fructose and starch on aorta collagen in the rat. (Preliminary Note). Journal of Atherosclerosis Research 8: 371-375.

Constantinides, P.
1965 Experimental atherosclerosis. Amsterdam, The Netherlands, Elsevier Publishing Co.

Faber, M. and G. Møller-Hou
1952 The human aorta, Part 5 (Collagen and elastin in the normal and hypertensive aorta). Acta Pathologica et Microbologica Scandinavica 31: 377-382.

Fisher, H. and P. Griminger
1963 Aging and food restriction: changes in body composition and hydroxyproline content of selected tissues. Journal of Nutrition 80: 350-354.

Gan, J. C., P. V. Narashimha Murthy, C. W. Nichols, Jr. and I. L. Chaikoff
1967 Mucosubstances in the chicken aorta. Part 1. Changes with age in acid mucopolysaccharides, glycoproteins, collagen and elastin. Journal of Atherosclerosis Research 7: 629-645.

Grant, R. A.
1967 Content and distribution of aortic collagen, elastin and carbohydrate in different species. Journal of Atherosclerosis Research 7: 463-472.

Gresham, G. A. and A. N. Howard
1961 The histogenesis of the atherosclerotic 'fatty streak'. Journal of Atherosclerotic Research 1: 413-416.

Grollman, A., C. Ashworth and W. Suki
1963 Atherosclerosis in the chicken. Correlation of the arterial blood pressure and arterial changes in the chicken. Archives of Pathology 75: 618-626.

Gross, J.
1958 Studies on the formation of collagen. Journal of Experimental Medicine 107: 265-277.

Gross, J.
1959 On the significance of the soluble collagens. In Page, I. H., ed. Connective tissue, thrombosis and atherosclerosis. Pp. 77-95. New York, New York, Academic Press.

Harkness, M. L. R., R. D. Harkness and D. A. McDonald
1957 The collagen and elastin content of the arterial wall in the dog. Royal Society (London) Philosophical Transactions Series B, 146: 541.

Harrington, W. F. and P. H. von Hippel
1961 The structure of collagen and gelatin. Advances in Protein Chemistry 16: 1-138.

Hess, R.
1964 Evaluation of drugs active against experimental atherosclerosis. Advances in Lipid Research 2: 295-445.

Jackson, D. S. and J. P. Bentley
1960 On the significance of the extractible collagen. Journal of Biophysical and Biochemical Cytology 7: 37-41.

Kanabracki, E. I., I. G. Fels and D. Kaplan
1960 Calcium, cholesterol and collagen levels in human aortas. Journal of Gerontology 15: 383-387.

Kao, K. T., D. M. Hilker and T. H. McGavack
1961a Connective tissue. IV. Synthesis and turnover of proteins in tissues of rats. Society for Experimental Biology and Medicine Proceedings 106: 121-124.

Kao, K. T., D. M. Hilker and T. H. McGavack
1961b Connective tissue. V. Comparison of synthesis and turnover of collagen and elastin in tissues of rats at several ages. Society for Experimental Biology and Medicine Proceedings 106: 335-338.

Katz, L. N. and R. Pick
1961 Experimental atherosclerosis as observed in the chicken. Journal of Atherosclerosis Research 1: 93-100.

Katz, L. N. and J. Stamler
1953 Experimental atherosclerosis. Springfield, Illinois, Charles C. Thomas.

Kurland, G. S. and A. S. Freedberg
1960 Hormones, cholesterol and coronary atherosclerosis. Circulation 22: 464-473.

Lansing, A. I.
1954 Ciba Foundation. Colloqium on Aging 1:88. Original not available; cited in Partridge, S. M. 1962. Elastin. Advances in Protein Chemistry 17: 244.

Lansing, A. I.
1959 Elastic tissue. In Lansing, A. I., ed. The arterial wall. Pp. 136-160. Baltimore, Maryland, The Williams and Wilkins Co.

Lansing, A. I., M. Alex and T. B. Rosenthal
1950 Calcium and elastin in human arteriosclerosis. Journal of Gerontology 5: 112-119.

Laufer, A., H. Ungar and M. Freund
1962 A microscopic study of coronary atherosclerosis in Israel. Journal of Atherosclerotic Research 2: 270-275.

Lowry, O. H., N. J. Rosebough, A. L. Farr and R. J. Randall
1951 Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193: 265-275.

Lowther, D. A.
1963 Chemical aspects of collagen fibrillogenesis. In Hall, D. A., ed. International review of connective tissue research. Vol. 1. Pp. 64-125. New York, New York, Academic Press.

McGavack, T. H. and K. T. Kao
1960 The influence of age and sex on the soluble collagen, insoluble collagen and elastin of rat tissues. Experimental Medicine and Surgery 18: 104-123.

Meyer, K. P. Hoffman and A. Linker
1959 Chemistry of ground substances. In Page, I. H., ed. Connective tissue, thrombosis and atherosclerosis. Pp. 181-191. New York, New York, Academic Press.

Mitchell, J. R. A. and C. J. Schwartz
1965 Arterial disease. Philadelphia, Pennsylvania, F. A. Davis Co.

Muir, H.
1964 Chemistry and metabolism of connective tissue glycoaminoglycans (mucopolysaccaharides). In Hall, D. A., ed. International review of connective tissue research. Vol. 2. Pp. 101-154. New York, New York, Academic Press.

Neuman, R. E. and M. A. Logan
1950 The determination of collagen and elastin in tissues. Journal of Biological Chemistry 186: 549-556.

Nichols, E. L.
1963 Effects of cholesterol and fat source and serum cholesterol and blood pressure levels in the growing chick. Unpublished Ph.D. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.

Noble, N. L., R. J. Boucek and K. T. Kao
1957 Biochemical observations of human atherosclerosis. Analysis of aortic intima. Circulation 15: 366-372.

Partridge, S. M.
1962 Elastin. Advances in Protein Chemistry 17: 227-302.

Pernis, B. and Clerici, E.
1957 Carbohydrates, collagen and elastin of the normal aortic wall and atherosclerotic hyaline plaques. Experientia 13: 351-353.

Pincus, G., ed.
1959 Hormones and atherosclerosis. New York, New York, Academic Press.

Prockop, D. J. and S. Undenfriend
1960 A specific method for the analysis of hydroxyproline in tissues and urine. Analytical Biochemistry 1: 228-239.

Ramachandran, G. N.
1963 Molecular structure of collagen. In Hall, D. A., ed. International review of connective tissue research. Vol. 1. Pp. 127-183. New York, New York, Academic Press.

Rose, R. J.
1967 Physiological responses of cockerels to dietary protein and energy restriction. Unpublished Ph.D. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.

Rossi, G. G., M. Mancini, P. Oriente, A. Vecchione, R. Vecchione, R. Cerqua and M. Cuzzupoli
1965 Comparitative studies on serum lipids and histochemical features of the arterial wall in animal species differently susceptible to experimental atherosclerosis. Journal of Atherosclerosis Research 5: 569-579.

Sandler, M. and G. H. Bourne, eds.
1963 Atherosclerosis and its origin. New York, New York, Academic Press.

Schmitt, F. O.
1959 The molecular basis of collagen structure. In Page, I. H., ed. Connective tissue, thrombosis and atherosclerosis. Pp. 43-65. New York, New York, Academic Press.

Smiley, J. D. and M. Ziff
1962 The metabolism of connective tissue. Annual Review of Medicine 13: 287-300.

Smith, E. B.
1965 The influence of age and atherosclerosis on the chemistry of the aorta intima. Part 2. Collagen and mucopolysaccharides. Journal of Atherosclerosis Research 5: 241-248.

Speers, G. M.
1965 Effect of protein and energy on blood pressure and atherosclerosis in the laying hen. Unpublished M.S. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.

Stamler, J.
1963 The relationship of sex and gonadal hormones to atherosclerosis. In Sandler, M. and G. H. Bourne, eds. Atherosclerosis and its origin. Pp. 231-262. New York, New York, Academic Press.

Sturkie, P. D., W. Durfee and M. Sheanan
1957 Demonstration of an improved method for measuring blood pressure in birds. Poultry Science 36: 1160-1161.

Taylor, H. E.
1953 The role of mucopolysaccharides in the pathogenesis of intimal fibrosis and atherosclerosis of human aorta. American Journal of Pathology 29: 871-883.

Verzar, F.
1964 Aging of the collagen fiber. In Hall, D. A., ed. International review of connective tissue research. Vol. 2. Pp. 244-300. New York, New York, Academic Press.

Weiss, H. S. and H. Fisher
1959 Evaluation of sex and segment differences in spontaneous avian aortic arteriosclerosis. American Journal of Physiology 197: 1219-1222.

Weissman, G. and S. Weissman
1960 X-ray diffraction studies of human aortic elastin residues. Journal of Clinical Investigation 39: 1657-1666.

Weissman, N. G. S. Shields and W. H. Carnes
1963 Cardiovascular studies on copper-deficient swine. IV. Content and solubility of the aortic elastin, collagen and hexosamine. Journal of Biological Chemistry 238: 3115-3118.

Wissler, R. W.
1968 The arterial medial cell, smooth muscle or multifunctional mesenchyme? Journal of Atherosclerosis Research 8: 201-213.

Woerner, C. A.
1959 Vasa vasorum of arteries, their demonstration and distribution. In Lansing, A. I., ed. The arterial wall. Pp. 1-14. Baltimore, Maryland, The Williams and Wilkins Co.

Yu, S. Y. and H. T. Blumenthal
1963 The calcification of elastic fibers. I. Biochemical studies. Journal of Gerontology 18: 127-134.

Zugibe, F. T.
1963 Histochemical studies of human coronary atherosclerosis: comparison with aortic and cerebral atherogenesis. Circulation Research 13: 401-409.

Zugibe, F. T. and K. D. Brown
1960 Histochemical studies in atherogenesis: human aorta. Circulation Research 8: 287-295.

Zugibe, F. T. and K. D. Brown
1961 Histochemical studies in atherogenesis: human cerebral arteries. Circulation Research 9: 897-905.

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APPENDIX

Table 23. Coefficients used in orthogonal planned comparisons. Experiment 709

Comparison	Treatments				
	20-3300	10-2200	10-2200 + Chol.	10-3300	10-3300 + Chol.
Protein	+4	-1	-1	-1	-1
Energy		-1	-1	+1	+1
Cholesterol		-1	+1	-1	+1
Energy x cholesterol		+1	-1	-1	+1

Table 24. Analysis of variance of serum cholesterol data. Experiment 709

Source of variation	d.f.	m.s.	F
Treatments	4	10,790,672	76.14**
Protein	1	10,078,534	71.07**
Energy	1	4,600,579	32.44**
Cholesterol	1	38,364,825	270.55**
Energy x cholesterol	1	5,070,399	35.76**
Error (a)	71	141,803	
Age	6	1,622,952	76.14**
Linear	1	4,594,014	215.54**
Quadratic	1	992,809	46.58**
Lack of fit	4	1,039,722	48.78**
Treatments x age	24	685,153	32.14**
Error (b)	261	21,315	

**Indicates significance at $P \leq 0.01$.

Table 25. Analysis of variance of systolic blood pressure. Experiment 709

Source of variation	d.f.	m.s.	F
Treatments	4	11,708	14.73**
Protein	1	38,908	48.94**
Energy	1	12,956	16.30**
Cholesterol	1	901	1.13
Energy x cholesterol	1	2	
Error (a)	71	795	
Age	6	786	2.38*
Linear	1	4,695	14.23**
Quadratic	1	728	2.21
Lack of fit	4	0	
Treatments x age	24	1,498	4.54**
Error (b)	261	330	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 26. Analysis of variance of total liver lipid data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	28.3	1.03
Linear	1	0.2	0.01
Deviation from linear	1	42.1	1.31
Treatments	4	1231.1	44.96**
Protein	1	368.3	11.45**
Energy	1	988.2	30.72**
Cholesterol	1	4390.0	136.50**
Energy x cholesterol	1	314.7	9.79**
Age x treatments	8	66.2	2.42*
Error	57	27.4	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 27. Analysis of variance of thoracic aorta scores. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	0.4185	3.89*
Linear	1	0.7657	5.58*
Deviation from linear	1	0.1271	0.93
Treatments	4	1.7167	15.95**
Protein	1	0.7695	5.61*
Energy	1	0.8336	6.07*
Cholesterol	1	3.7182	27.09**
Energy x cholesterol	1	0.7452	5.43*
Age x treatments	8	0.3487	3.24**
Error	57	0.1076	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 28. Analysis of variance of abdominal aorta scores. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	5.414	7.55**
Linear	1	11.532	15.45**
Deviation from linear	1	0.153	0.21
Treatments	4	5.094	7.10**
Protein	1	1.138	1.52
Energy	1	1.506	2.02
Cholesterol	1	12.329	16.51**
Energy x cholesterol	1	3.720	4.98*
Age x treatments	8	0.956	1.33
Error	57	0.717	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 29. Analysis of variance of thoracic aorta total lipid data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	40.987	1.00
Linear	1	41.500	1.06
Deviation from linear	1	37.680	0.97
Treatments	4	20.885	0.51
Protein	1	1.909	0.05
Energy	1	25.602	0.66
Cholesterol	1	49.765	1.28
Energy x cholesterol	1	11.165	0.29
Age x treatments	8	25.027	0.61
Error	57	40.936	

Table 30. Analysis of variance of abdominal aorta total lipid data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	114.30	5.89**
Linear	1	61.60	3.33
Deviation from linear	1	155.79	8.43**
Treatments	4	35.04	1.81
Protein	1	34.36	1.86
Energy	1	19.57	1.06
Cholesterol	1	65.22	3.53
Energy x cholesterol	1	12.23	0.66
Age x treatments	8	11.81	0.61
Error	57	19.41	

**Indicates significance at $P \leq 0.01$.

Table 31. Analysis of variance of thoracic aorta buffer extract protein data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	3458.2	26.42**
Linear	1	6981.6	52.30**
Deviation from linear	1	106.8	0.80
Treatments	4	786.5	6.01**
Protein	1	1443.6	10.81**
Energy	1	1618.3	12.12**
Cholesterol	1	76.7	0.57
Energy x cholesterol	1	26.5	0.20
Age x treatments	8	152.1	1.16
Error	57		

**Indicates significance at $P \leq 0.01$.

Table 32. Analysis of variance of abdominal aorta buffer extract protein data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	2348.9	7.45**
Linear	1	4188.8	14.18**
Deviation from linear	1	495.4	1.68
Treatments	4	1315.6	4.17**
Protein	1	1357.8	4.60*
Energy	1	1966.4	6.65*
Cholesterol	1	1002.0	3.39
Energy x cholesterol	1	822.9	2.79
Age x treatments	8	154.9	
Error	57	315.2	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 33. Analysis of variance of thoracic aorta buffer extract hydroxyproline data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	0.2692	10.42**
Linear	1	0.4573	18.78**
Deviation from linear	1	0.1672	6.87*
Treatments	4	0.0406	1.57
Protein	1	0.0948	3.89
Energy	1	0.0089	0.36
Cholesterol	1	0.0062	0.26
Energy x cholesterol	1	0.0290	1.19
Age x treatments	8	0.0138	0.53
Error	57	0.0258	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 34. Analysis of variance of abdominal aorta buffer extract hydroxyproline data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	0.4850	3.46*
Linear	1	0.9433	6.32*
Deviation from linear	1	0.2655	1.78
Treatments	4	0.0699	0.50
Protein	1	0.0805	0.54
Energy	1	0.0032	0.02
Cholesterol	1	0.0115	0.08
Energy x cholesterol	1	0.0377	0.25
Age x treatments	8	0.2143	1.53
Error	57	0.1401	

*Indicates significance at $P \leq 0.05$.

Table 35. Analysis of variance of thoracic aorta autoclave extract protein. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	19279	17.97**
Linear	1	50	0.04
Deviation from linear	1	32712	25.86**
Treatments	4	610	0.57
Protein	1	84	0.07
Energy	1	55	0.04
Cholesterol	1	200	0.16
Energy x cholesterol	1	680	0.54
Age x treatments	8	2633	2.45*
Error	57	1073	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 36. Analysis of variance of abdominal aorta autoclave extract protein data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	68731	49.27**
Linear	1	8887	6.75*
Deviation from linear	1	132007	100.31**
Treatments	4	375	0.27
Protein	1	792	0.60
Energy	1	66	0.05
Cholesterol	1	0	0.00
Energy x cholesterol	1	204	0.15
Age x treatments	8	752	0.54
Error	57	1395	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 37. Analysis of variance of thoracic aorta autoclave extract hydroxyproline data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	114.87	9.66**
Linear	1	61.22	4.94*
Deviation from linear	1	129.27	10.43**
Treatments	4	11.66	0.98
Protein	1	0.07	0.01
Energy	1	0.26	0.02
Cholesterol	1	5.72	0.46
Energy x cholesterol	1	17.12	1.38
Age x treatments	8	15.96	1.34
Error	57	11.90	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 38. Analysis of variance of abdominal aorta autoclave extract hydroxyproline data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	324.75	7.73**
Linear	1	32.28	0.78
Deviation from linear	1	608.98	14.66**
Treatments	4	9.46	0.23
Protein	1	2.29	0.06
Energy	1	4.17	0.10
Cholesterol	1	13.43	0.32
Energy x cholesterol	1	17.02	0.41
Age x treatments	8	38.08	0.91
Error	57	42.02	

**Indicates significance at $P \leq 0.01$.

Table 39. Analysis of variance of thoracic aorta elastin residue data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	109.62	5.90**
Linear	1	4.00	0.19
Deviation from linear	1	161.70	7.77**
Treatments	4	44.32	2.39
Protein	1	31.40	1.51
Energy	1	7.14	0.34
Cholesterol	1	4.87	0.23
Energy x cholesterol	1	95.60	4.59*
Age x treatments	8	36.79	1.98
Error	57	18.56	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 40. Analysis of variance of abdominal aorta elastin residue data. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	74.524	1.21
Linear	1	35.256	0.60
Deviation from linear	1	60.222	1.02
Treatments	4	82.481	1.34
Protein	1	33.863	0.58
Energy	1	135.165	2.30
Cholesterol	1	29.381	0.50
Energy x cholesterol	1	77.120	1.31
Age x treatments	8	40.972	0.67
Error	57	61.357	

Table 41. Analysis of variance of thoracic aorta insoluble/soluble collagen ratios. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	1286.7	7.21**
Linear	1	588.8	3.50
Deviation from linear	1	1941.0	11.54**
Treatments	4	276.1	1.55
Protein	1	537.1	3.19
Energy	1	48.4	0.29
Cholesterol	1	76.2	0.45
Energy x cholesterol	1	456.1	2.71
Age x treatments	8	95.5	0.54
Error	57	178.3	

**Indicates significance at $P \leq 0.01$.

Table 42. Analysis of variance of abdominal aorta insoluble/soluble collagen ratios. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	103.61	11.91**
Linear	1	15.97	1.69
Deviation from linear	1	240.38	25.49**
Treatments	4	2.69	0.31
Protein	1	6.10	0.65
Energy	1	2.48	0.26
Cholesterol	1	0.20	0.02
Energy x cholesterol	1	0.04	0.00
Age x treatments	8	14.62	1.68
Error	57	8.70	

**Indicates significance at $P \leq 0.01$.

Table 43. Analysis of variance of thoracic aorta elastin residue/collagen ratios. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	15.760	9.23**
Linear	1	12.375	7.00*
Deviation from linear	1	15.489	8.76**
Treatments	4	1.824	1.07
Protein	1	0.003	0.00
Energy	1	0.254	0.14
Cholesterol	1	0.824	0.47
Energy x cholesterol	1	4.494	2.54
Age x treatments	8	2.192	1.28
Error	57	1.708	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 44. Analysis of variance of abdominal aorta elastin residue/collagen ratios. Experiment 709

Source of variation	d.f.	m.s.	F
Age	2	0.4988	3.37*
Linear	1	0.0336	0.24
Deviation from linear	1	0.8151	5.76*
Treatments	4	0.1012	0.68
Protein	1	0.0328	0.23
Energy	1	0.1688	1.19
Cholesterol	1	0.0013	0.01
Energy x cholesterol	1	0.0967	0.65
Age x treatments	8	0.0967	0.65
Error	57	0.1478	

*Indicates significance at $P \leq 0.05$.

Table 45. Correlation coefficients[a] within treatments for aorta scores, aorta analysis data, terminal blood pressure and terminal serum cholesterol level. Experiment 709

	Variables						
	1	2	3	4	5	6	7
1	1.00[b]	.10	-.05	.17	-.13	.18	.17
2		1.00	-.16	.04	-.04	.32	.04
3			1.00	-.27	.08	-.75	-.59
4				1.00	-.32	.06	-.18
5					1.00	.03	.07
6						1.00	.60
7							1.00
8							
9							
10							
11							
12							
13							
14							
15							
16							

[a]Correlation coefficients must differ from zero by + or - 0.23 or by + or - 0.30 to be significant at $P < 0.05$ and $P < 0.01$ respectively.

[b]Variables as follows:
1 Thoracic segment aorta score
2 Thoracic segment percent lipid
3 Thoracic segment percent elastin residue
4 Thoracic segment buffer extract protein

Table 45 (Continued)

	Variables								
	8	9	10	11	12	13	14	15	16
1	.26[b]	-.04	-.07	.08	.13	-.02	.06	-.15	.04
2	.14	.18	-.18	-.00	.07	.24	.29	-.14	.01
3	.16	-.01	.46	.04	.04	-.39	-.29	-.18	.05
4	.29	.16	-.16	.44	-.11	-.20	-.10	-.07	.03
5	-.12	-.04	.24	-.37	.10	-.01	-.06	-.00	.17
6	-.11	.25	-.44	.04	.02	.60	.41	.15	-.00
7	-.24	.13	-.43	-.18	.16	.47	.36	.06	-.10
8	1.00	.22	-.13	.40	-.00	-.11	.12	-.26	.07
9		1.00	-.20	.42	.03	.30	.28	-.08	.03
10			1.00	-.30	-.38	-.40	-.54	.02	.06
11				1.00	.01	.04	.08	-.10	.05
12					1.00	.21	.41	-.11	.08
13						1.00	.72	.05	.12
14							1.00	-.16	.08
15								1.00	.04
16									1.00

[b]Variables as follows:
8 Abdominal segment aorta score
9 Abdominal segment percent lipid
10 Abdominal segment percent elastin residue
11 Abdominal segment buffer extract protein
12 Abdominal segment buffer extract hydroxyproline
13 Abdominal segment autoclave extract protein
14 Abdominal segment autoclave extract hydroxyproline
15 Terminal systolic blood pressure
16 Terminal serum cholesterol level.

Table 46. Analysis of variance of serum cholesterol data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	10,818,275	32.42**
Oil (O)	1	25,736	0.08
Cholesterol (C)	1	24,483,240	73.36**
P x O	1	45,008	0.13
P x C	1	10,434,095	31.27**
O x C	1	530,680	1.59
P x O x C	1	284,278	0.85
Error (a)	63	333,728	
Age (A)	6	1,749,931	20.92**
P x A	6	732,708	8.76**
E x A	6	105,320	1.26
C x A	6	1,605,251	19.19**
Error (b)	256	83,642	

**Indicates significance at $P \leq 0.01$.

Table 47. Analysis of variance of total liver lipid data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	967	56.01**
Soybean oil (O)	1	325	18.85**
Cholesterol (C)	1	12203	707.00**
Age (A)	2	150	8.69**
P x O	1	167	9.67**
P x C	1	297	17.19**
P x A	2	18	1.02
O x C	1	609	35.29**
O x A	2	30	1.76
C x A	2	192	11.11**
P x O x C	1	5	0.28
P x O x A	2	1	0.06
P x C x A	2	17	0.99
O x C x A	2	28	1.60
Error	41	17	

**Indicates significance at $P \leq 0.01$.

Table 48. Analysis of variance of thoracic aorta scores. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	5.1086	19.60**
Soybean oil (O)	1	2.5565	9.81**
Cholesterol (C)	1	7.6906	29.51**
Age (A)	2	0.0624	0.24
P x O	1	1.4105	5.41*
P x C	1	5.8793	22.56**
P x A	2	0.1575	0.60
O x C	1	2.2897	8.78**
O x A	2	0.8529	3.27*
C x A	2	0.1004	0.39
P x O x C	1	1.1885	4.56*
P x O x A	2	0.6107	2.34
P x C x A	2	0.2250	0.86
O x C x A	2	0.5808	2.23
Error	41	0.2606	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 49. Analysis of variance of abdominal aorta scores. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	4.8266	8.28**
Soybean oil (O)	1	2.3748	4.07*
Cholesterol (C)	1	9.9291	17.02**
Age (A)	2	3.0699	5.26**
P x O	1	0.0330	0.06
P x C	1	5.4322	9.31**
P x A	2	0.9044	1.55
O x C	1	1.3657	2.34
O x A	2	0.4749	0.81
C x A	2	0.3124	0.54
P x O x C	1	0.0788	0.14
P x O x A	2	0.1606	0.28
P x C x A	2	0.1678	0.29
O x C x A	2	1.3587	2.33
Error	41	0.5832	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 50. Analysis of variance of thoracic aorta total lipid data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	12.790	16.02**
Soybean oil (O)	1	6.122	7.67**
Cholesterol (C)	1	22.700	28.43**
Age (A)	2	4.430	5.55*
P x O	1	0.556	0.70
P x C	1	13.286	16.64**
P x A	2	0.614	0.77
O x C	1	0.240	0.30
O x A	2	6.508	8.15**
C x A	2	0.701	0.88
P x O x C	1	4.612	5.78*
P x O x A	2	1.780	2.23
P x C x A	2	0.458	0.57
O x C x A	2	5.992	7.50**
Error	41	0.799	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 51. Analysis of variance of abdominal aorta total lipid data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	157.14	19.34**
Soybean oil (O)	1	8.33	1.03
Cholesterol (C)	1	107.88	13.28**
Age (A)	2	101.88	12.54**
P x O	1	3.74	0.46
P x C	1	36.96	4.55*
P x A	2	2.82	0.35
O x C	1	11.39	1.40
O x A	2	14.40	1.77
C x A	2	8.68	1.07
P x O x C	1	28.47	3.50
P x O x A	2	3.90	0.48
P x C x A	2	2.97	0.37
O x C x A	2	5.88	0.72
Error	41	8.12	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 52. Analysis of variance of thoracic buffer extract protein data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	3.40	0.05
Soybean oil (O)	1	0.02	0.00
Cholesterol (C)	1	279.48	3.84
Age (A)	2	368.71	5.06*
P x O	1	42.89	0.59
P x C	1	151.79	2.08
P x A	2	3.37	0.05
O x C	1	36.24	0.50
O x A	2	78.01	1.07
C x A	2	338.69	4.65*
P x O x C	1	82.35	1.13
P x O x A	2	105.36	1.45
P x C x A	2	40.42	0.55
O x C x A	2	162.09	2.22
Error	41	72.86	

*Indicates significance at $P \leq 0.05$.

Table 53. Analysis of variance of abdominal buffer extract protein data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	4.0	0.02
Soybean oil (O)	1	28.4	0.14
Cholesterol (C)	1	267.4	1.36
Age (A)	2	146.1	0.75
P x O	1	65.2	0.33
P x C	1	1241.6	6.34*
P x A	2	264.0	1.35
O x C	1	37.2	0.19
O x A	2	112.8	0.58
C x A	2	164.4	0.84
P x O x C	1	40.9	0.21
P x O x A	2	119.2	0.61
P x C x A	2	41.7	0.21
O x C x A	2	137.3	0.70
Error	41	195.9	

*Indicates significance at $P \leq 0.05$.

Table 54. Analysis of variance of thoracic aorta buffer extract hydroxyproline data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	0.0117	10.06**
Soybean oil (O)	1	0.0007	0.56
Cholesterol (C)	1	0.0048	4.14*
Age (A)	2	0.0285	24.57**
P x O	1	0.0028	2.39
P x C	1	0.0010	0.92
P x A	2	0.0020	1.75
O x C	1	0.0000	0.03
O x A	2	0.0027	2.36
C x A	2	0.0024	2.05
P x O x C	1	0.0004	0.31
P x O x A	2	0.0049	4.22
P x C x A	2	0.0023	2.01
O x C x A	2	0.0012	1.06
Error	41	0.0012	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 55. Analysis of variance of abdominal aorta buffer extract hydroxyproline data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	0.0000	0.00
Soybean oil (O)	1	0.0000	0.00
Cholesterol (C)	1	0.0216	1.37
Age (A)	2	0.0472	2.99
P x O	1	0.0207	1.31
P x C	1	0.0074	0.47
P x A	2	0.0007	0.05
O x C	1	0.0048	0.30
O x A	2	0.0038	0.24
C x A	2	0.0306	1.94
P x O x C	1	0.0241	1.52
P x O x A	2	0.0040	0.25
P x C x A	2	0.0121	0.77
O x C x A	2	0.0058	0.37
Error	41	0.0158	

Table 56. Analysis of variance of thoracic aorta autoclave extract protein data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	50.9	0.05
Soybean oil (O)	1	18.4	0.02
Cholesterol (C)	1	1930.3	1.75
Age (A)	2	3945.1	3.58*
P x O	1	16.3	0.01
P x C	1	336.2	0.31
P x A	2	2333.1	2.12
O x C	1	1959.2	1.78
O x A	2	632.7	0.57
C x A	2	292.2	0.27
P x O x C	1	36.2	0.03
P x O x A	2	1290.1	1.17
P x C x A	2	1630.2	1.48
O x C x A	2	1072.0	0.97
Error	41	1102.4	

*Indicates significance at $P \leq 0.05$.

Table 57. Analysis of variance of abdominal aorta autoclave extract protein data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	346.9	0.23
Soybean oil (O)	1	1021.8	0.68
Cholesterol (C)	1	187.0	0.12
Age (A)	2	7674.7	5.10*
P x O	1	77.2	0.05
P x C	1	2564.9	1.71
P x A	2	2805.3	1.86
O x C	1	14.3	0.01
O x A	2	2582.6	1.72
C x A	2	507.3	0.34
P x O x C	1	1230.4	0.82
P x O x A	2	1898.8	1.26
P x C x A	2	1960.3	1.30
O x C x A	2	3866.7	2.57
Error	41	1504.3	

*Indicates significance at $P \leq 0.05$.

Table 58. Analysis of variance of thoracic aorta autoclave extract hydroxyproline data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	19.04	3.56
Soybean oil (O)	1	13.58	2.54
Cholesterol (C)	1	0.40	0.08
Age (A)	2	139.91	26.19**
P x O	1	0.05	0.01
P x C	1	10.89	2.04
P x A	2	3.04	0.57
O x C	1	4.34	0.81
O x A	2	8.66	1.62
C x A	2	20.32	3.80*
P x O x C	1	1.92	0.36
P x O x A	2	6.43	1.20
P x C x A	2	0.11	0.02
O x C x A	2	3.61	0.68
Error	41	5.34	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 59. Analysis of variance of abdominal aorta autoclave extract hydroxyproline data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	117.45	2.87
Soybean oil (O)	1	36.91	0.90
Cholesterol (C)	1	101.83	2.49
Age (A)	2	458.55	11.20**
P x O	1	14.99	0.37
P x C	1	4.76	0.12
P x A	2	42.88	1.05
O x C	1	1.23	0.03
O x A	2	74.28	1.81
C x A	2	28.31	0.69
P x O x C	1	3.45	0.08
P x O x A	2	81.20	1.98
P x C x A	2	55.88	1.37
O x C x A	2	5.22	0.13
Error	41	40.93	

**Indicates significance at $P \leq 0.01$.

Table 60. Analysis of variance of thoracic aorta elastin residue data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	74.65	2.56
Soybean oil (O)	1	98.57	3.38
Cholesterol (C)	1	29.35	1.01
Age (A)	2	87.61	3.00
P x O	1	43.98	1.51
P x C	1	1.74	0.06
P x A	2	6.58	0.23
O x C	1	18.55	0.64
O x A	2	101.53	3.48*
C x A	2	0.07	0.00
P x O x C	1	6.00	0.21
P x O x A	2	6.37	0.22
P x C x A	2	14.40	0.49
O x C x A	2	6.78	0.23
Error	41	29.19	

*Indicates significance at $P \leq 0.05$.

Table 61. Analysis of variance of abdominal aorta elastin residue data. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	1.03	0.06
Soybean oil (O)	1	0.73	0.04
Cholesterol (C)	1	1.76	0.10
Age (A)	2	378.39	20.41**
P x O	1	3.67	0.20
P x C	1	59.11	3.19
P x A	2	14.50	0.78
O x C	1	0.00	0.00
O x A	2	2.45	0.13
C x A	2	0.97	0.05
P x O x C	1	37.06	2.00
P x O x A	2	1.28	0.07
P x C x A	2	0.05	0.00
O x C x A	2	39.21	2.11
Error	41	18.54	

**Indicates significance at $P \leq 0.01$.

Table 62. Analysis of variance of thoracic aorta insoluble/soluble collagen ratio. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	1002.2	1.22
Soybean oil (O)	1	1032.7	1.26
Cholesterol (C)	1	3465.4	4.16*
Age (A)	2	32278.8	39.43**
P x O	1	1051.4	1.28
P x C	1	660.0	0.81
P x A	2	815.0	1.00
O x C	1	64.9	0.08
O x A	2	952.5	1.16
C x A	2	19.0	0.02
P x O x C	1	14.4	0.02
P x O x A	2	978.6	1.20
P x C x A	2	1331.5	1.63
O x C x A	2	312.2	0.38
Error	41	818.6	

*Indicates significance at $P \leq 0.05$.

**Indicates significance at $P \leq 0.01$.

Table 63. Analysis of variance of abdominal aorta insoluble/soluble collagen ratio. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	306.8	2.00
Soybean oil (O)	1	121.5	0.79
Cholesterol (C)	1	11.6	0.08
Age (A)	2	2052.4	13.37**
P x O	1	166.7	1.09
P x C	1	37.3	0.24
P x A	2	90.7	0.59
O x C	1	30.5	0.20
O x A	2	379.0	2.47
C x A	2	189.0	1.23
P x O x C	1	256.7	1.67
P x C x A	2	113.1	0.74
P x C x A	2	180.7	1.18
O x C x A	2	86.2	0.56
Error	41	153.6	

**Indicates significance at $P \leq 0.01$.

Table 64. Analysis of variance of thoracic aorta elastin residue/collagen ratio. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	1.0661	3.49
Soybean oil (O)	1	0.0194	0.06
Cholesterol (C)	1	0.1249	0.41
Age (A)	2	5.0522	16.54**
P x O	1	0.1121	0.37
P x C	1	0.2268	0.74
P x A	2	0.0723	0.24
O x C	1	0.2197	0.72
O x A	2	1.1423	3.74
C x A	2	0.3545	1.16
P x O x C	1	0.0532	0.17
P x O x A	2	0.1258	0.41
P x C x A	2	0.0509	0.17
O x C x A	2	0.0444	0.15
Error	41	0.3054	

**Indicates significance at $P \leq 0.01$.

Table 65. Analysis of variance of abdominal aorta elastin residue/collagen ratio. Experiment 727

Source of variation	d.f.	m.s.	F
Protein (P)	1	0.0756	1.55
Soybean oil (O)	1	0.0014	0.03
Cholesterol (C)	1	0.0328	0.67
Age (A)	2	0.9280	18.96**
P x O	1	0.0006	0.01
P x C	1	0.0629	1.29
P x A	2	0.0682	1.39
O x C	1	0.0057	0.12
O x A	2	0.0295	0.60
C x A	2	0.0101	0.21
P x O x C	1	0.0341	0.70
P x O x A	2	0.0198	0.40
P x C x A	2	0.0273	0.56
O x C x A	2	0.0632	1.29
Error	41	0.0489	

**Indicates significance at $P \leq 0.01$.

Table 66. Correlation coefficients[a] for aorta score, aorta analysis data, and terminal serum cholesterol level. Experiment 727

	Variables						
	1	2	3	4	5	6	7
1	1.00[b]	.40	-.24	.18	-.21	-.13	.11
2		1.00	-.11	.50	-.12	.06	-.40
3			1.00	-.33	.24	-.43	-.47
4				1.00	.24	.25	-.34
5					1.00	-.03	-.50
6						1.00	.18
7							1.00
8							
9							
10							
11							
12							
13							
14							
15							

[a]Correlation coefficients must differ from zero by + or - 0.25 or by + or - 0.32 to be significant at $P < 0.05$ or $P < 0.01$ respectively.

[b]Variables numbered as follows:
1 Thoracic segment aorta score
2 Thoracic segment percent lipid
3 Thoracic segment percent elastin residue
4 Thoracic segment buffer extract protein
5 Thoracic segment buffer extract hydroxyproline
6 Thoracic segment autoclave extract protein
7 Thoracic segment autoclave extract hydroxyproline.

Footnote continued on following page.

Table 66 (Continued)

	Variables							
	8	9	10	11	12	13	14	15
1	.47[b]	.34	-.04	.17	-.30	-.16	-.21	.42
2	.16	.54	.13	.09	.02	.07	-.20	.62
3	-.26	-.00	.39	.02	.11	-.20	-.27	.10
4	-.05	.43	.03	.26	.16	.04	-.24	.45
5	-.18	.30	.35	.07	.24	.06	-.32	.12
6	.17	-.02	-.37	.11	-.01	.47	.26	-.23
7	.30	-.37	-.48	-.11	-.08	.08	.53	-.32
8	1.00	.03	-.42	.12	-.28	.12	.17	.11
9		1.00	.34	.11	-.01	-.16	-.38	.41
10			1.00	-.21	.13	-.54	-.56	.18
11				1.00	.14	.15	-.16	.22
12					1.00	.15	.15	.05
13						1.00	.48	.04
14							1.00	-.21
15								1.00

[b]Variables numbered as follows:
8 Abdominal segment aorta score
9 Abdominal segment percent lipid
10 Abdominal segment percent elastin residue
11 Abdominal segment buffer extract protein
12 Abdominal segment buffer extract hydroxyproline
13 Abdominal segment autoclave extract protein
14 Abdominal segment autoclave extract hydroxyproline
15 Terminal serum cholesterol level.